JDS International Seminar 2019

Antibiotics and Antibiotic Resistance Genes in a Coastal Area: Spatial Distribution and Potential Risks

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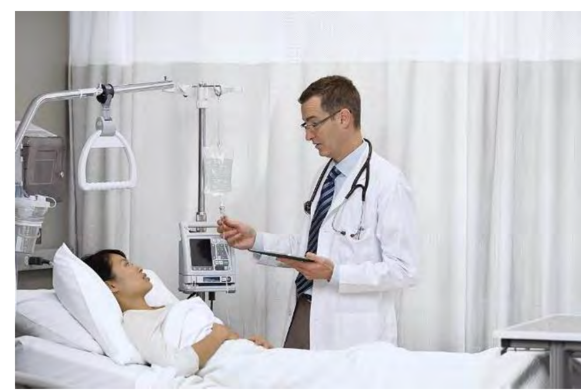
- **Background**
- Study Plan
- Antibiotics and Risks
- ARGs and Risks
- Conclusions

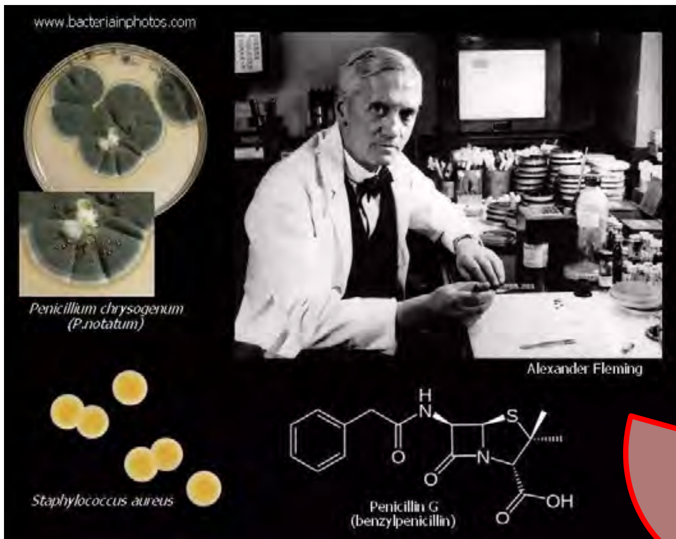
Global Demand for Medicines

- Population growth
- Health needs
- Economic development
- Market expansion



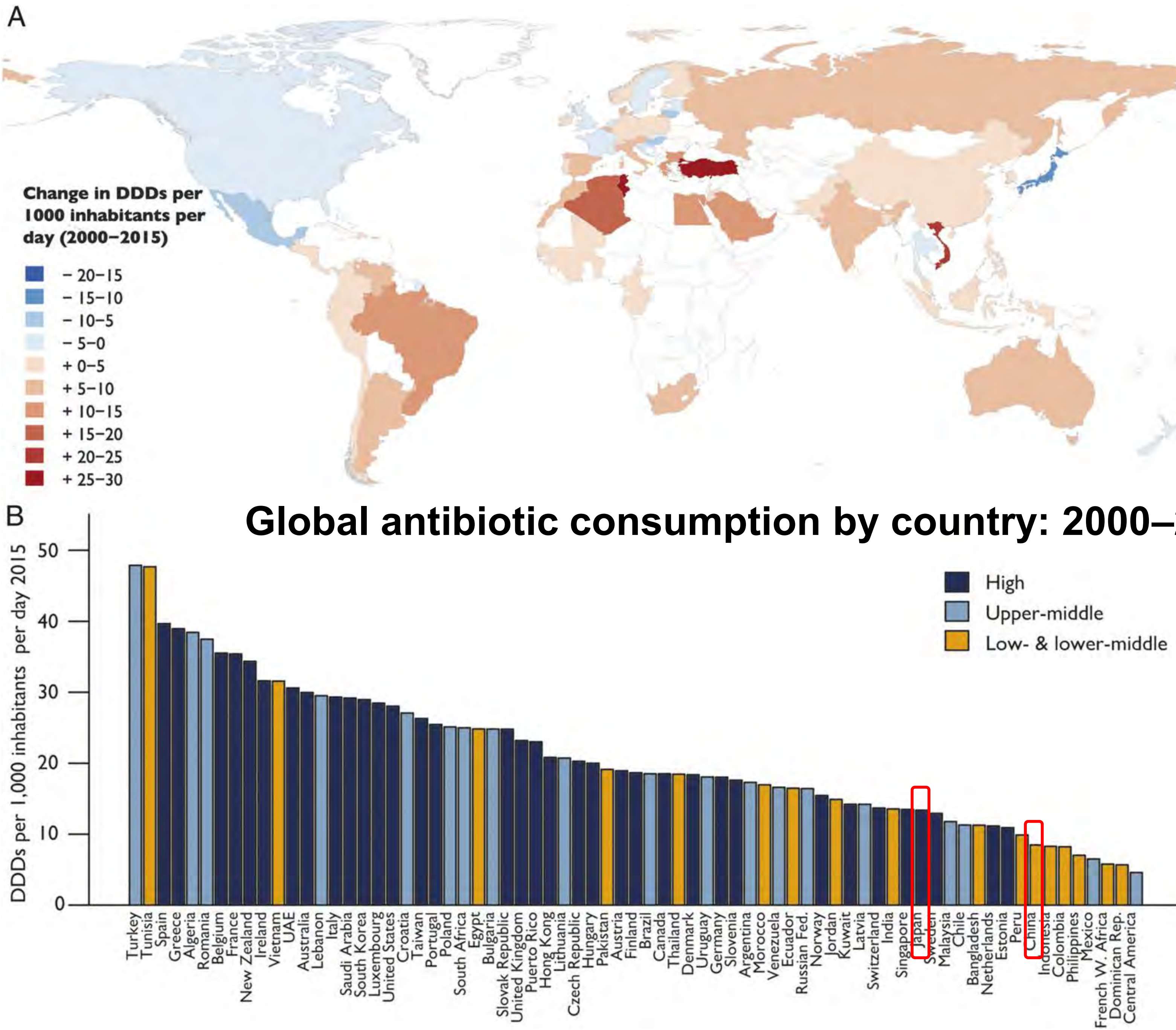
Antibiotics are a type of antimicrobial substances active against bacteria.

Antibiotic medications are widely used in the treatment and prevention of bacterial infections.



Class	Typical Antibiotic
Beta-lactams	penicillin, cephalosporin, carbapenem, amoxicillin, cephalexin, methicillin, …
Aminoglycosides	streptomycin, kanamycin, …
Macrolides	erythromycin, roxithromycin, clarithromycin, …
Amphenicols	chloramphenicol, thiamphenicol, …
Tetracyclines	tetracycline, terramycin, …
Lincosamides	lincomycin, clindamycin, pirlimycin, …
Sulfonamides	sulfadiazine, sulfamethoxazole, …
Quinolones	ciprofloxacin, pipemidic acid, moxifloxacin, levofloxacin, …
…	...

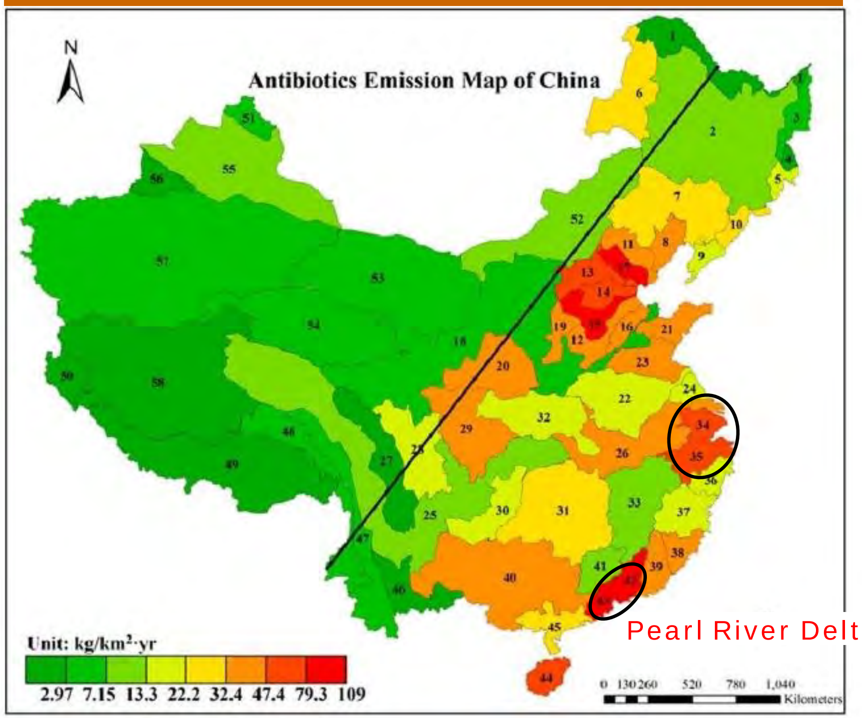
Global antibiotic consumption by country: 2000–2015



Klein et al., ***PNAS***, 2018.

Pathways of Antibiotics
from human society to natural environment

The emission density of 36 antibiotics in river basins of China:



Oberoi et al. ***Environmental Science & Technology***, 2019.

Zhang et al., ***Environmental Science & Technology***, 2015.

A Survey in a coastal city in Pearl River Delta:

Science of the Total Environment 636 (2018) 1009–1019

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

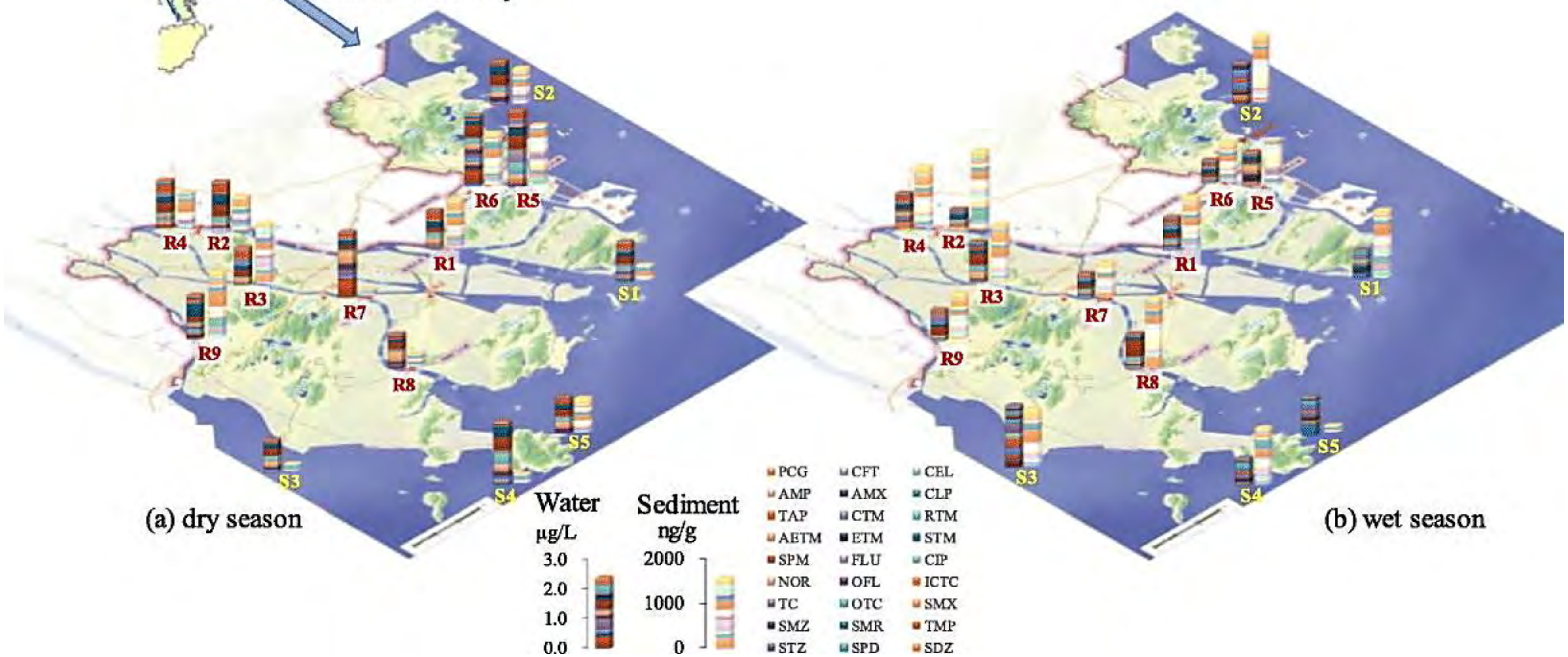
ELSEVIER

Antibiotics in water and sediments of rivers and coastal area of Zhuhai City, Pearl River estuary, south China

Si Li [a,b], Wanzi Shi [c], Huimin Li [a], Nan Xu [c], Ruijie Zhang [a], Xuejiao Chen [c], Weiling Sun [a,c,*], Donghui Wen [a], Shanliang He [d], Jianguo Pan [d], Zhidong He [d], Yingying Fan [d]

[a] College of Environmental Sciences and Engineering, Peking University, The Key Laboratory of Water and Sediment Sciences, Ministry of Education, Beijing 100871, China
[b] Xiamen Urban Water Environmental Eco-Planning and Remediation Engineering Research Center, Xiamen 361021, China
[c] Shenzhen Key Laboratory for Heavy Metal Pollution Control and Reutilization, School of Environment and Energy, Peking University Shenzhen Graduate School, Shenzhen 518055, China
[d] Zhuhai Environmental Protection Monitoring Station, Zhuhai 519000, Guangdong, China

Antibiotics Distribution



Potential Risks
of the antibiotics entering into environment

- Inhibit the growth and activities of microorganisms in the environment;
- Excert toxic effect on animals and plants, and disturb their ecological functions, thus may influence on the stabilization of ecosystem;
- Induce the evolution of antibiotic resistant bacteria (ARB), which usually carry antibiotic resistance genes (ARGs). And ARGs can propagate and spread in many environments, plants, animals as well as human beings.

Environmental risk assessment: $RQ = {}^{MEC}/_{PNEC}$

RQ: risk quotient;

MEC: measured environmental concentration;

PNEC: predicted no-effect concentration, $PNEC = {}^{EC_{50}(LC_{50})}/_{AF}$ **or** $PNEC = {}^{chv}/_{AF}$

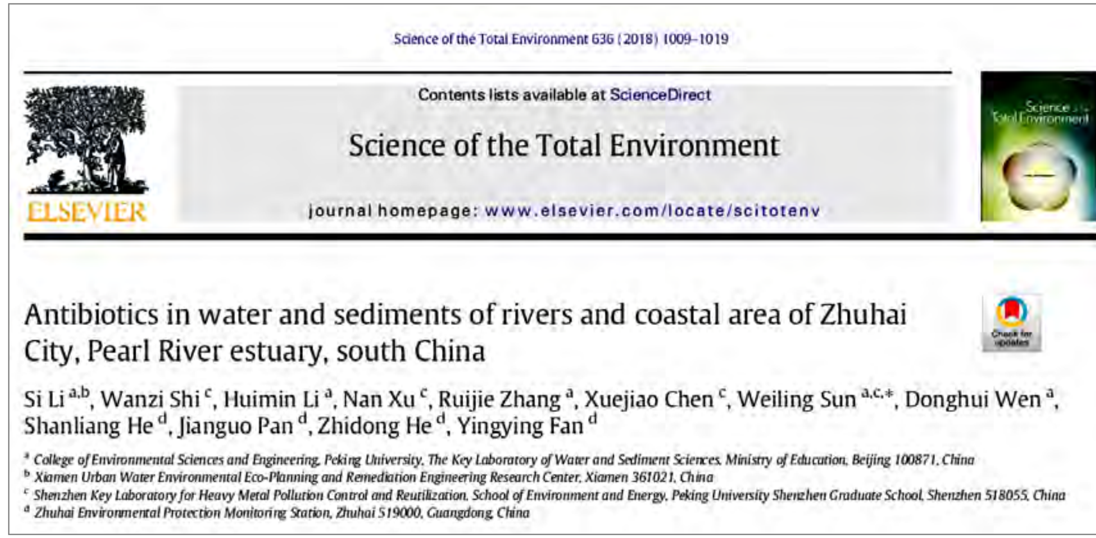
EC_{50}: half maximal effective concentration;

LC_{50}: median lethal concentration;

chv: chronic toxicity;

AF: assessment factor, 1000 and100 for acute and chronic toxicity, respectively, in freshwaters.

A Survey in a coastal city in Pearl River Delta:



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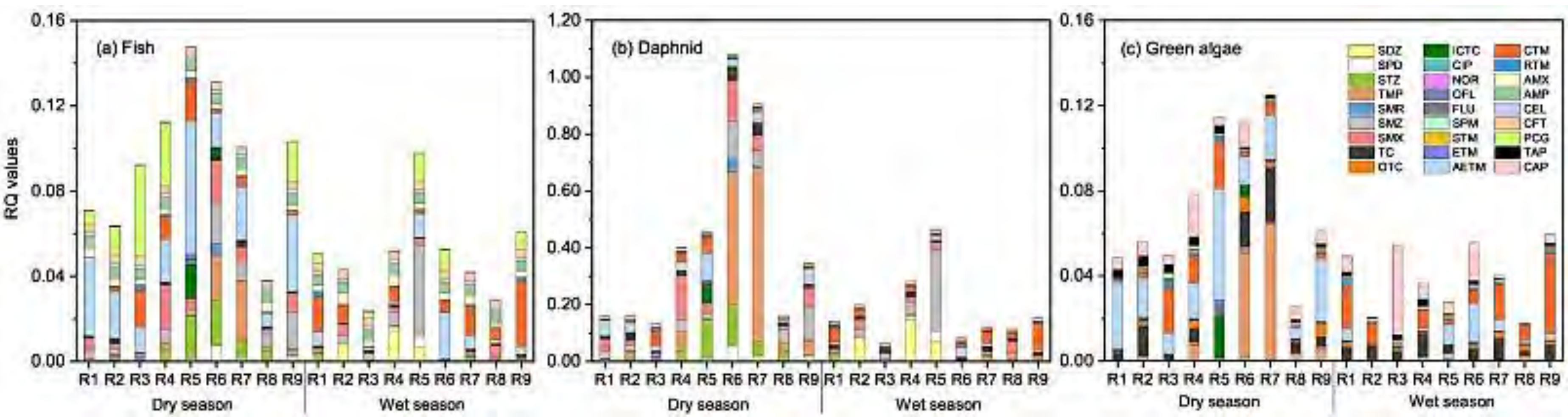
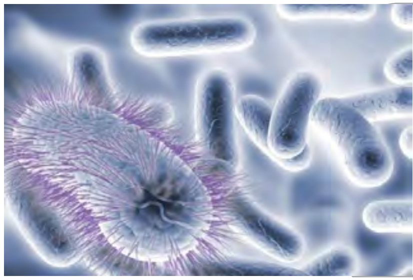
Risk Assessment



AETM, CTM, SMZ, SMX, and TMP greatly contributed to the ecological risk to different trophic levels of organisms in river and coastal waters.

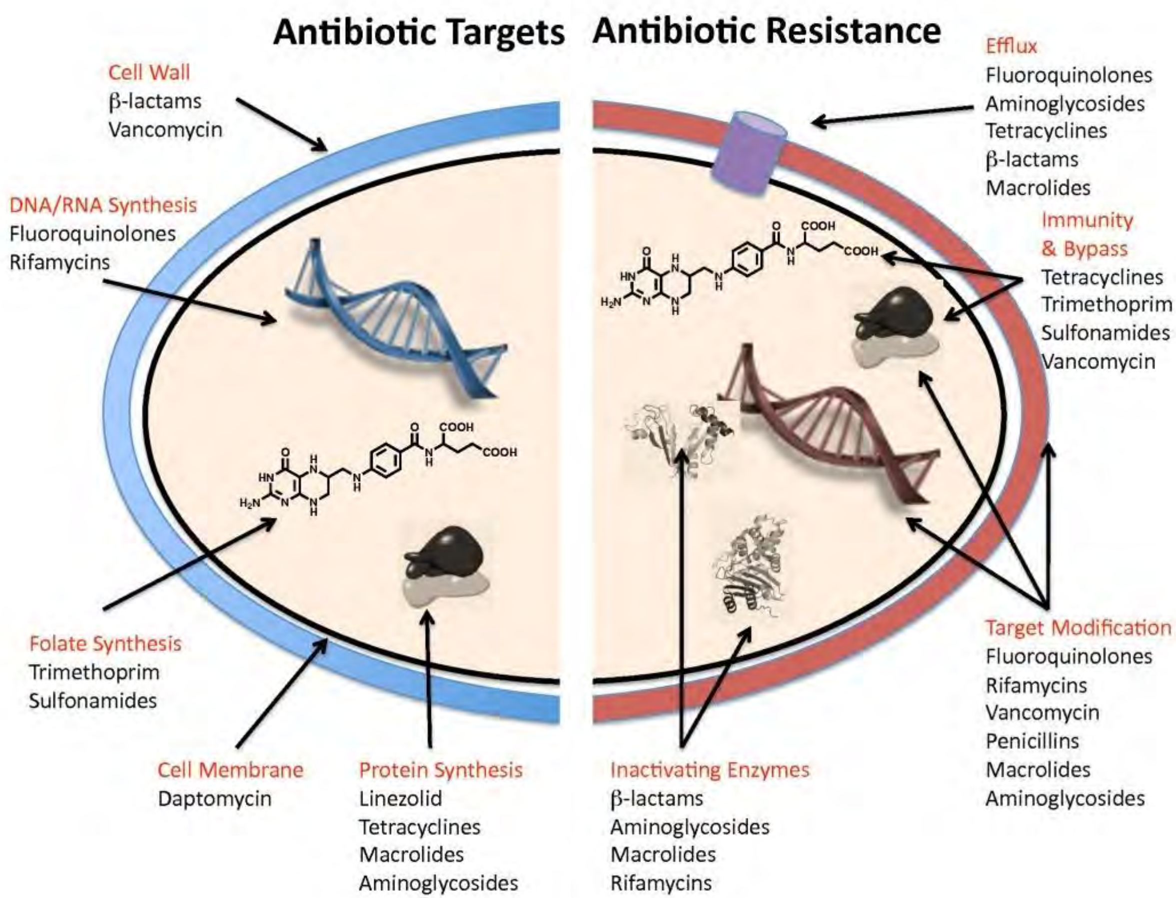


Antibiotic Resistance is the ability of a bacterium to resist the effects of antibiotics.

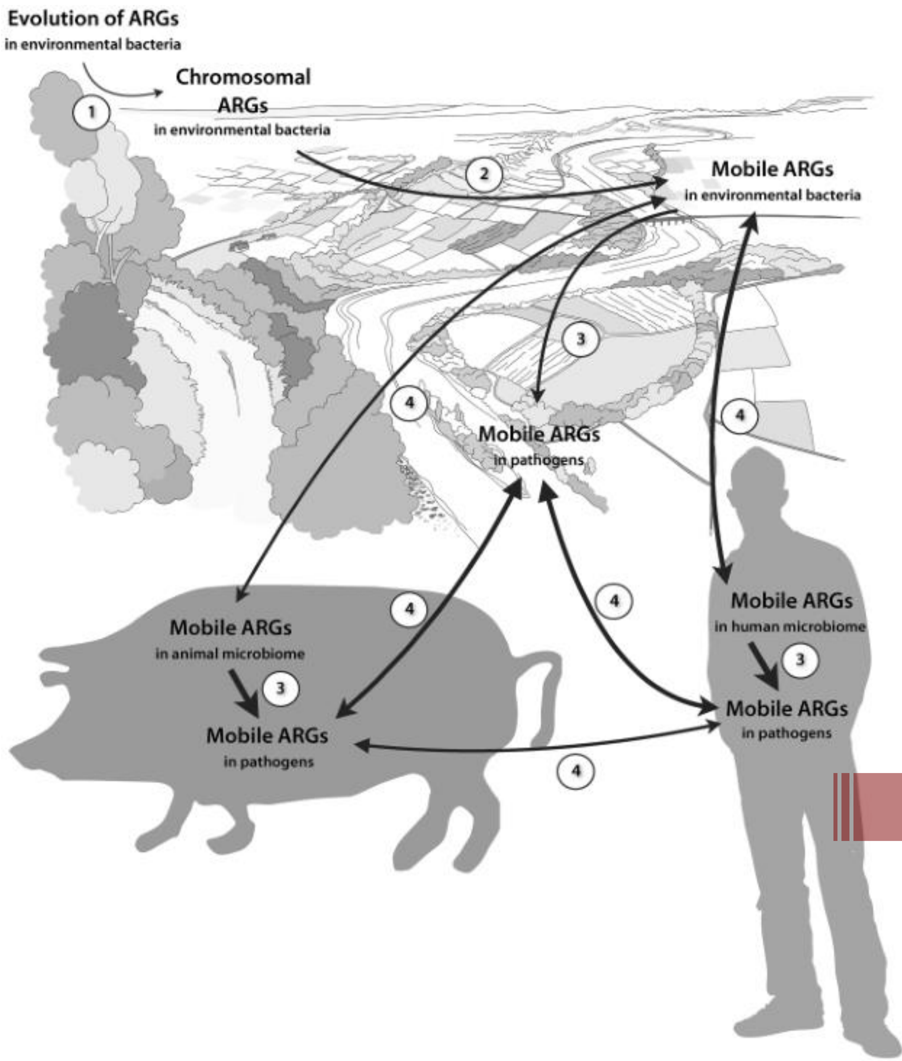
Antibiotic Resistance Genes are the DNA sequences which confer antibiotic resistance to one or several antibiotics.

Mechanism of Antibiotic Resistance:

1. Efflux pump
2. Decreased permeability
3. Target modification
4. Alteration of metabolic pathway
5. Enzymatic inactivation
6. …

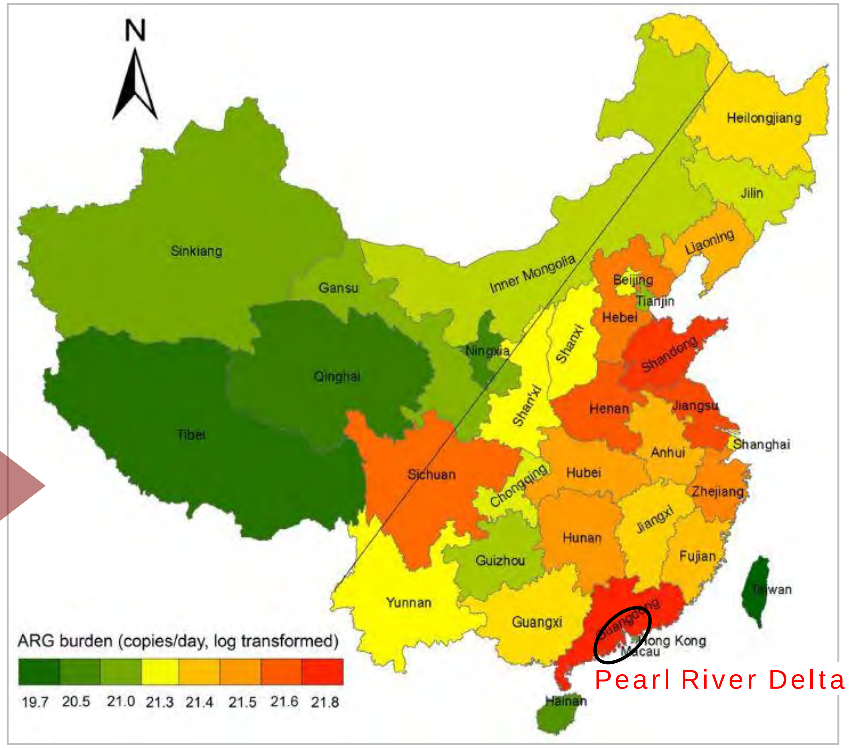


Occurrence of Antibiotic Resistance Genes



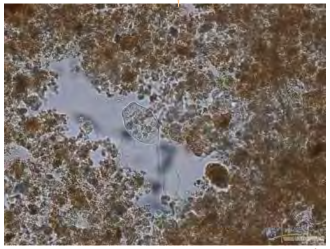
Bengtsson-Palme et al., *FEMS Microbiology Reviews*, 2018.

ARG burden based on urban populations of administrative districts in China:

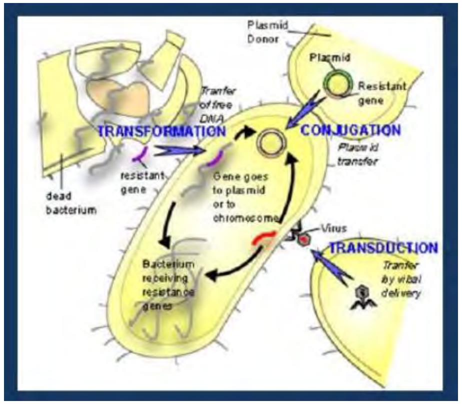


Su et al., *Microbiome*, 2017.

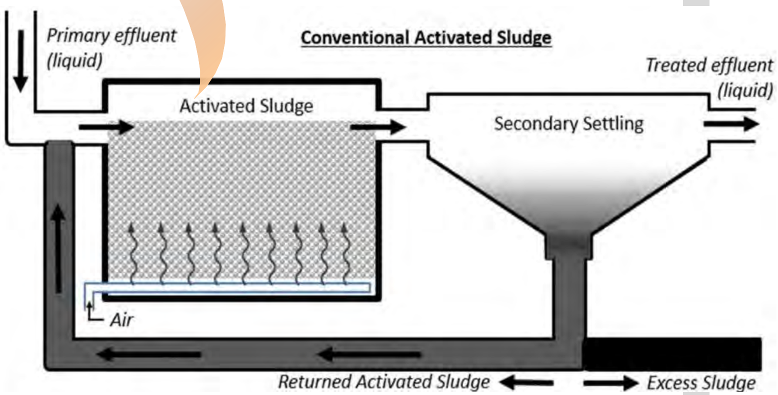
WWTP: land-based point source



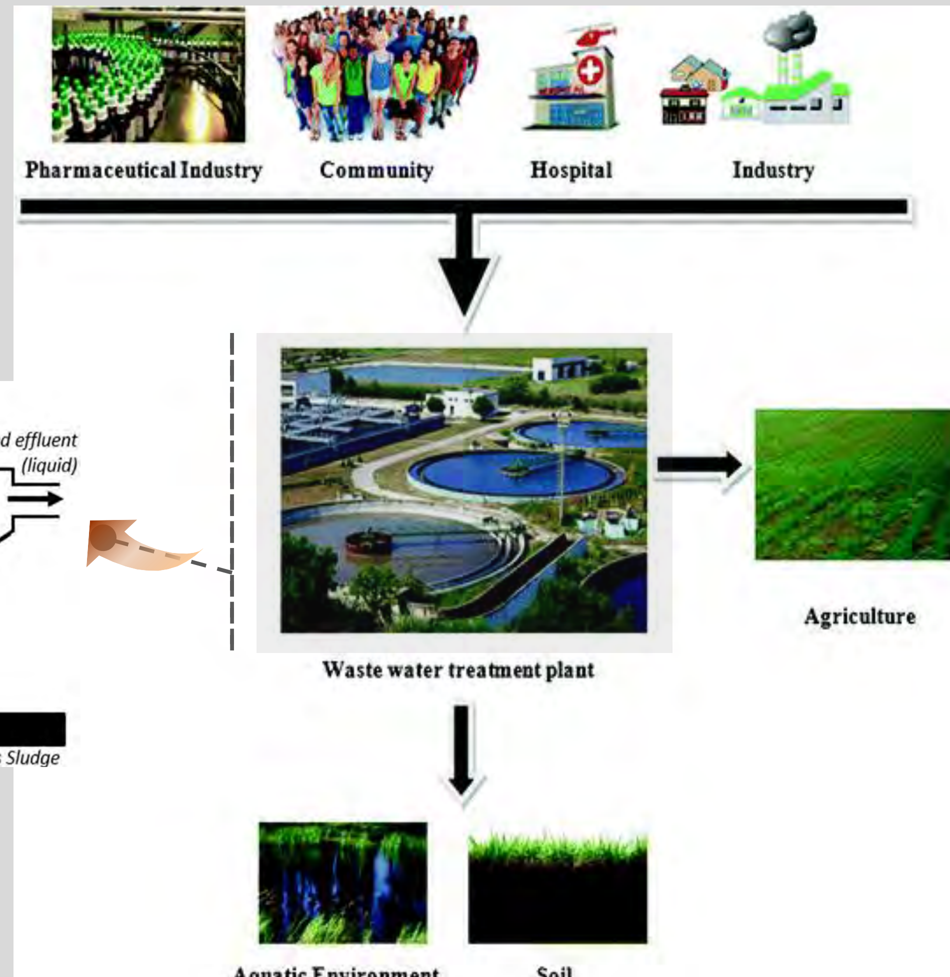
activated sludge



ARGs spreading



The role of wastewater treatment plant in the dissemination route of ARGs:



Tripathi V. and Tripathi P. **Antibiotic Resistance Genes: An Emerging Environmental Pollutant**. from: Kesari K. (eds) Perspectives in Environmental Toxicology. Environmental Science and Engineering. 2017.

A Survey in a coastal city in Pearl River Delta:

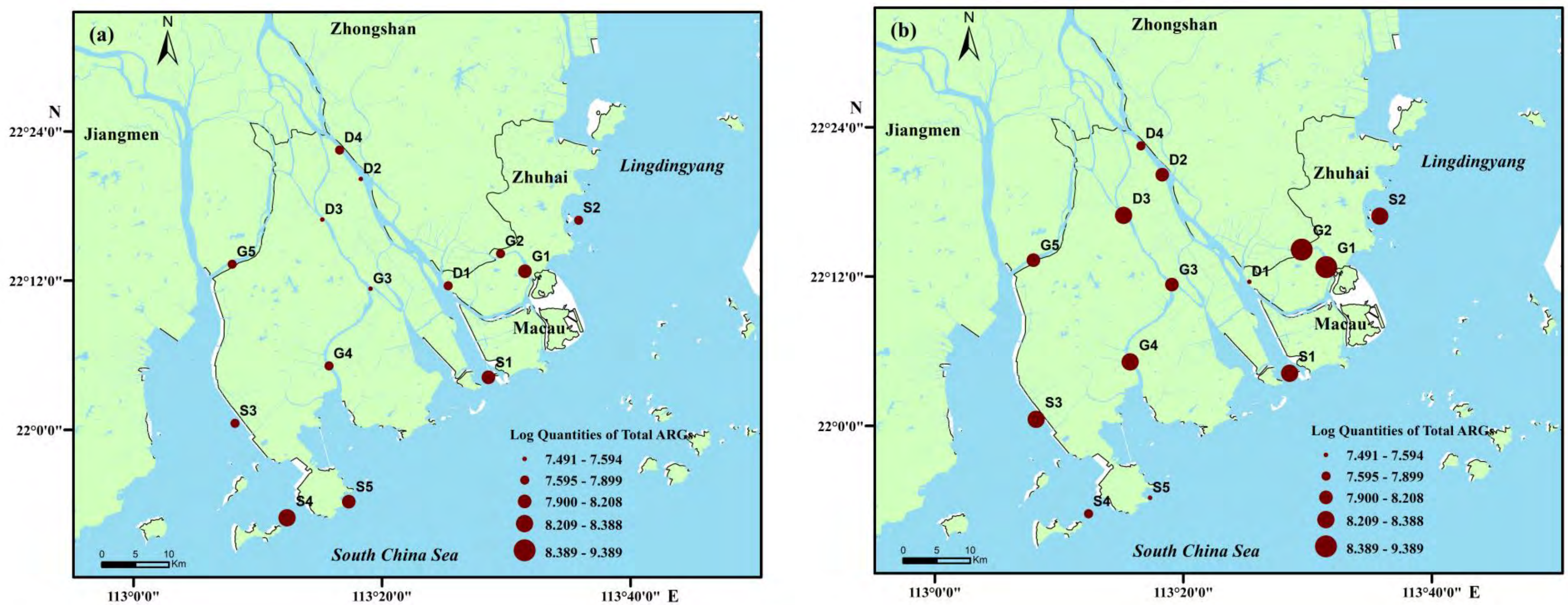
ARGs Distribution

Environmental Science and Pollution Research (2018) 25:26209–26217
https://doi.org/10.1007/s11356-018-2664-0

RESEARCH ARTICLE

Occurrence and distribution of antibiotic resistance genes in the sediments of drinking water sources, urban rivers, and coastal areas in Zhuhai, China

Aolin Li[1] · Lujun Chen[1,2] · Yan Zhang[3] · Yile Tao[4] · Hui Xie[1] · Si Li[4] · Weiling Sun[4] · Jianguo Pan[5] · Zhidong He[5] · Chaoan Mai[5] · Yingying Fan[5] · Huanchao Xian[5] · Zebin Zhang[5] · Donghui Wen[4]



Concentrations of total ARGs: drinking water sources << urban rivers ≅ nearby coastal area, indicating the different degrees of anthropogenic impacts and consequent health risks.

Potential Risks of ARGs

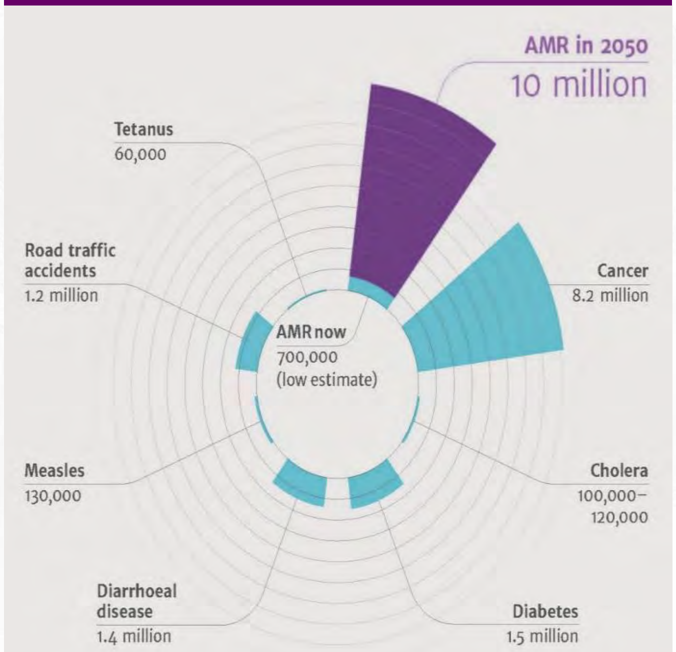
- Some human symbiotic microorganisms and pathogens could access to antibiotic resistance via the wide propagation and spread of ARGs in environments.
- A growing and worldwide healthcare crisis;
- A rapid rising cost in new drugs' R & D.

  The last-resort antibiotics, e.g., glycylcyclines, oxazolidinones, carbapenems, polymyxins, are severely restricted in hospitals.

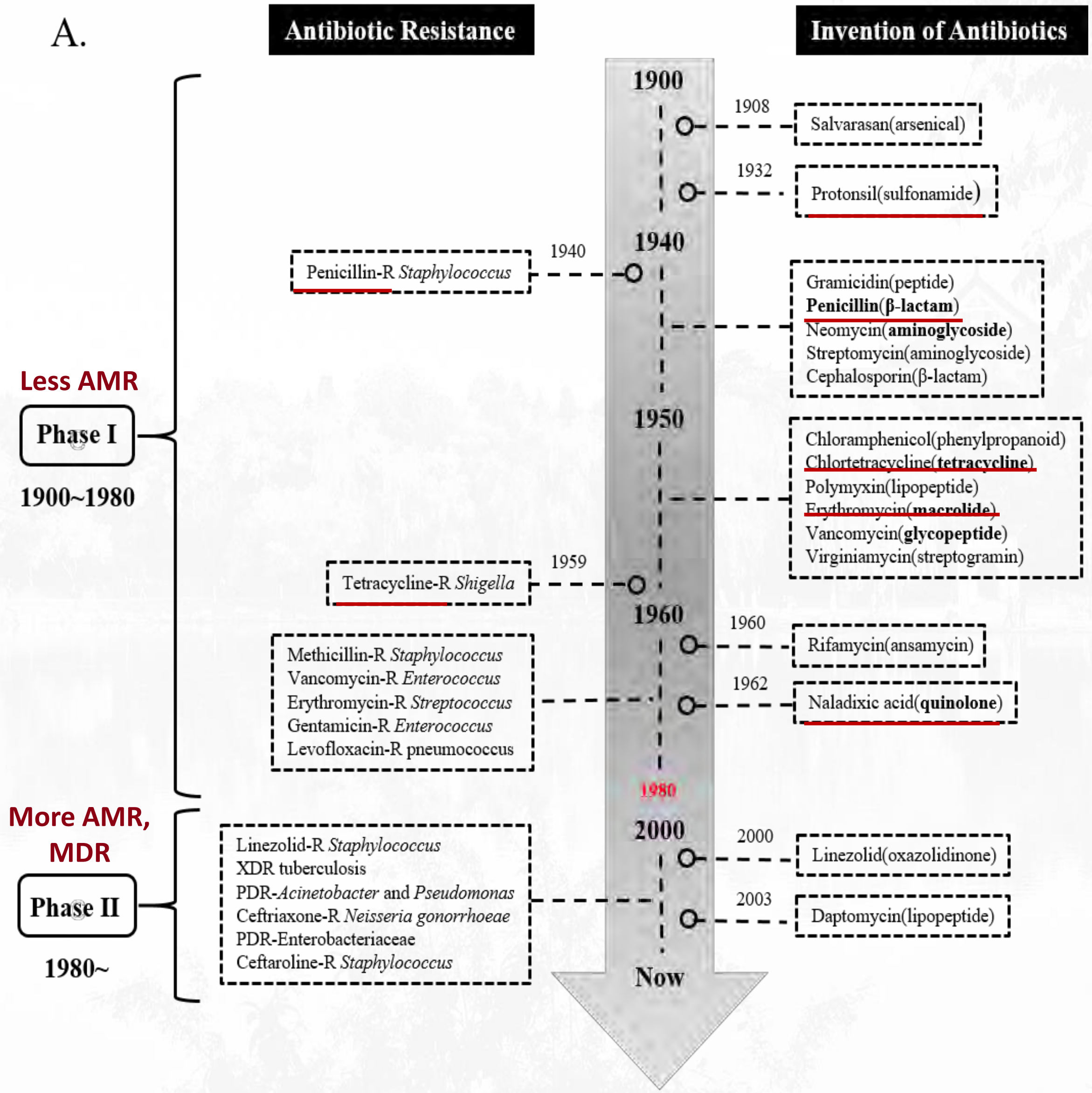
http://amr.ncgm.go.jp/general/1-3-1.html

A War between Microbes and Human Beings

Deaths attributable to antimicrobial resistance every year compared to other major causes of death:

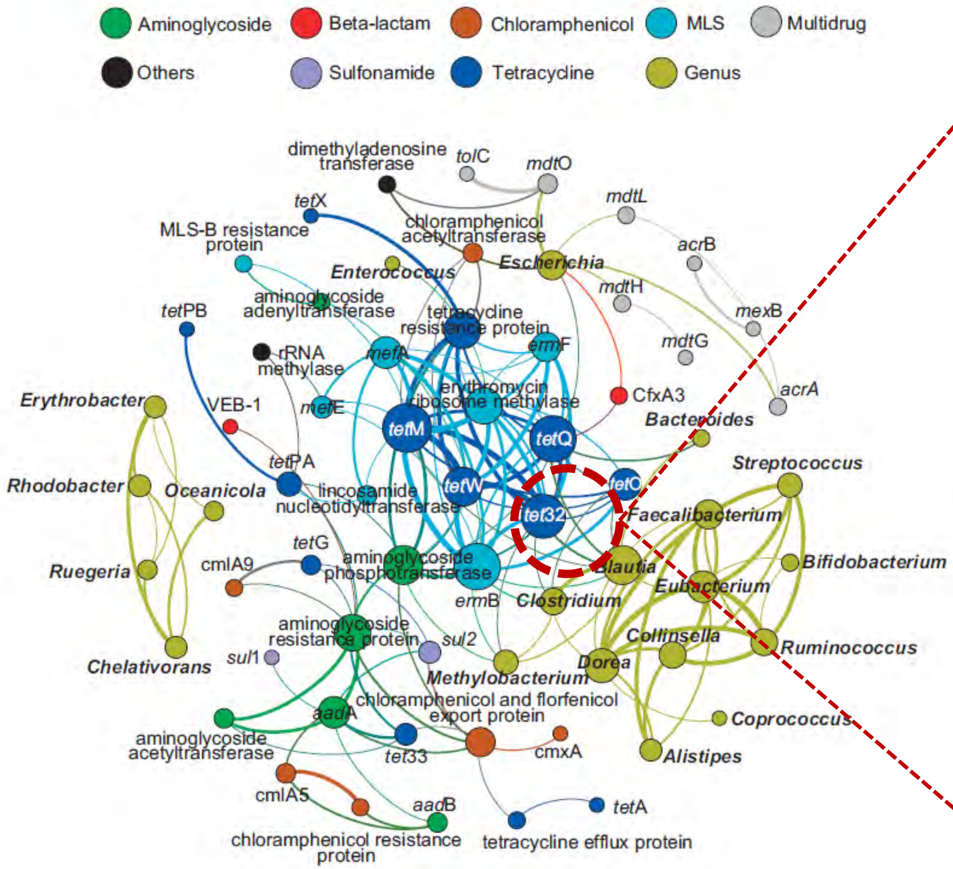


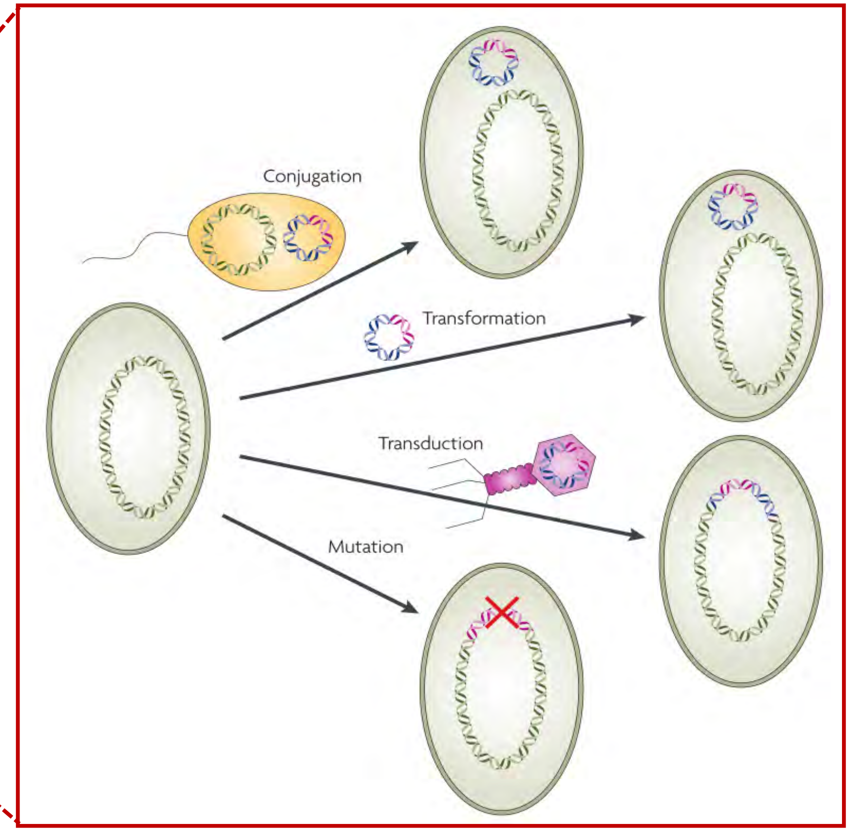
Jim O'Neill. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations, from **The Review on Antimicrobial Resistance.** 2014.



Su et al., ***Microbiology China***, 2018.

Dissemination of ARGs in Environment





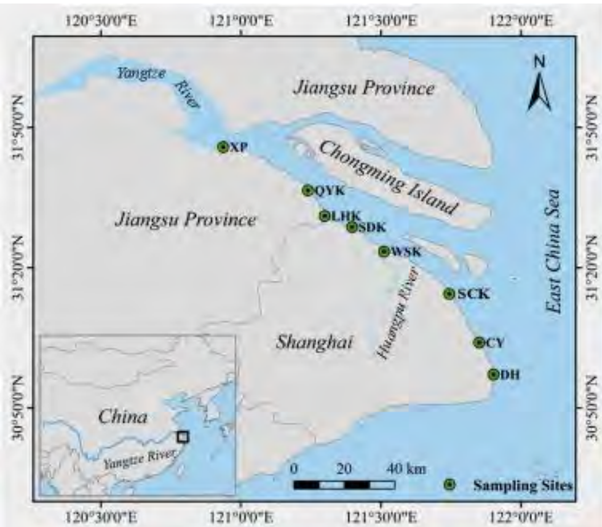
Co-occurrence network between **ARGs** and **microbial community**

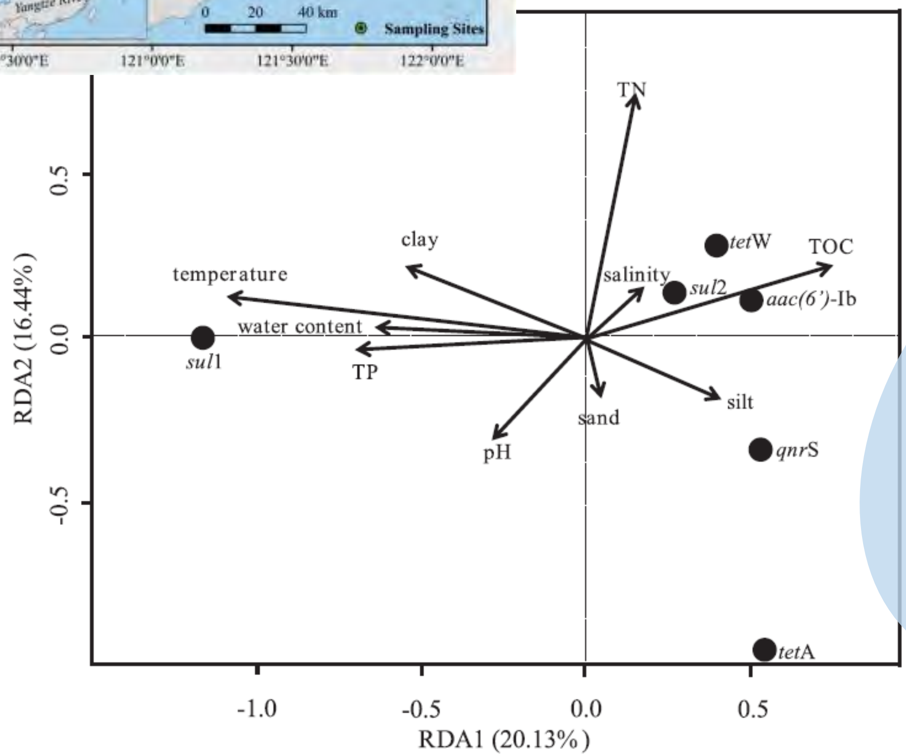
Horizontal gene transfer is crucial for ARGs spread in microbial community

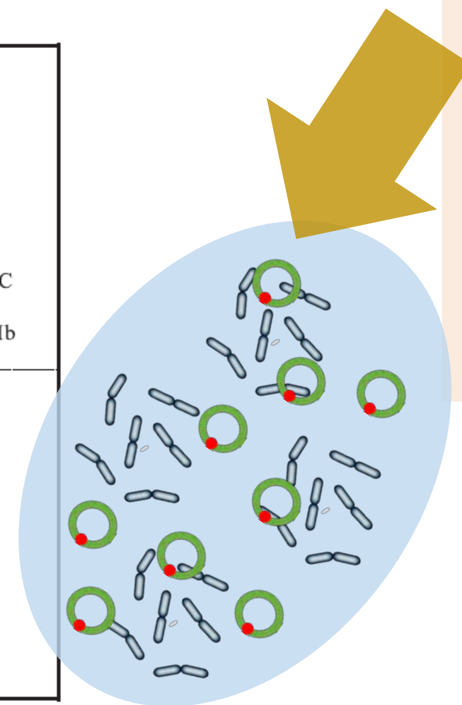
Li et al., ***The ISME Journal***, 2015.

Andersson and **Hughes**, ***Nature Reviews Microbiology***, 2010.

Environmental Factors on ARGs Dissemination







- pH
- Temperature
- **Nutrients**
- **Organic pollutants**
- **Heavy metals**
- Biocides
- Virulence factors
- **Antibiotics?**
- **Mobile genetic elements**
- ……

Guo et al., ***Environmental Pollution***, 2018.

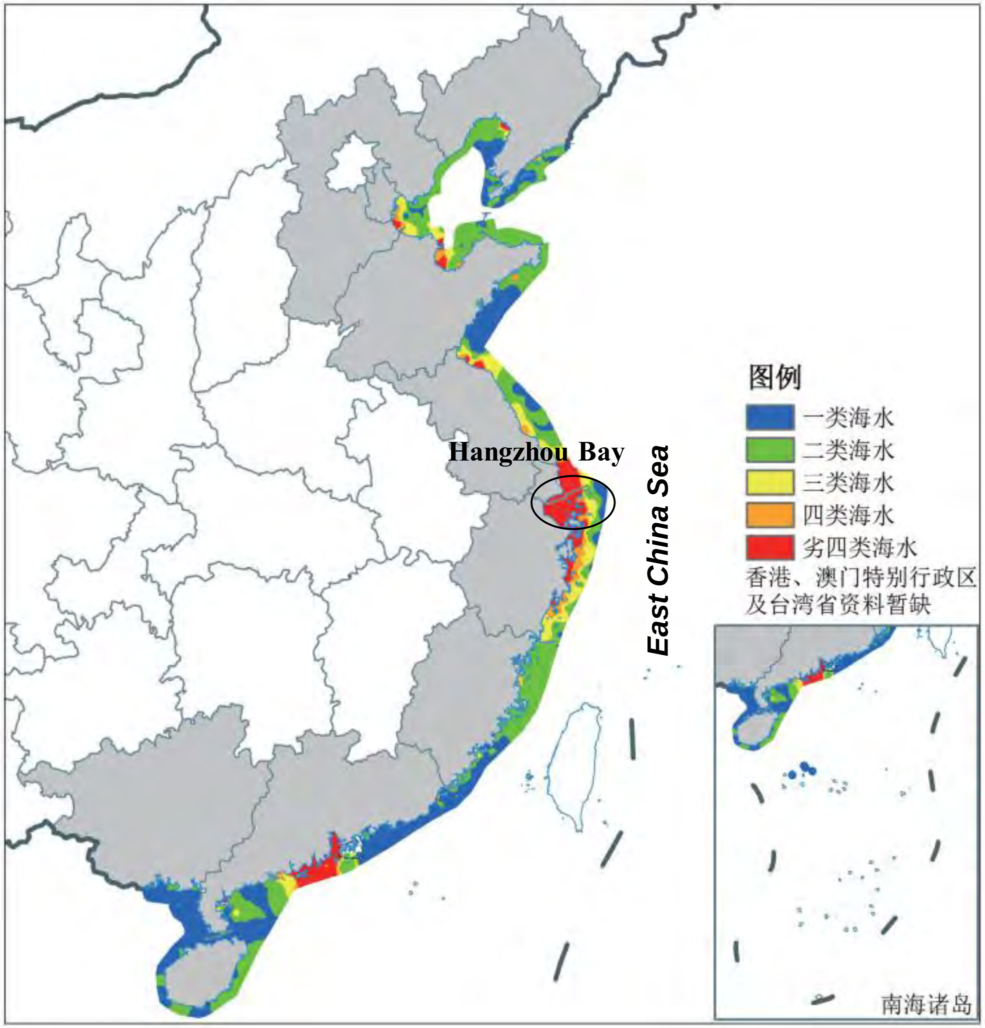
2

- Background
- **Study Plan**
- Antibiotics and Risks
- ARGs and Risks
- Conclusions

Our Research Progress

- **Comprehensive investigation of the study area**
  - ✓ Population, geographic and hydrographic information, …
  - ✓ Coastal environmental quality status
  - ✓ Local industrial activities
  - ✓ Land-based pollutants discharging into sea
- **Contamination of antibiotics**
  - ✓ Spatial distribution of antibiotics
  - ✓ The potential risks
  - ✓ Priority individual antibiotics
- **Occurrence of ARGs**
  - ✓ Spatial distribution of ARGs
  - ✓ ARGs in the microbial community network
  - ✓ The potential risks

Study Area: Hangzhou bay, East China Sea



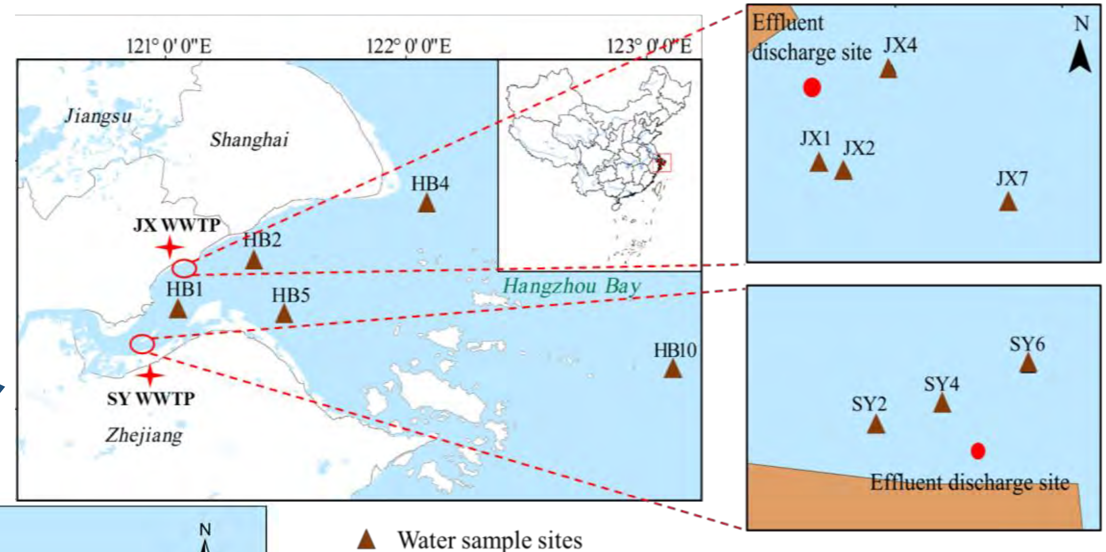
Water quality of China Seas in 2017 (Ministry of Ecology and Envrionment of China, 2018)

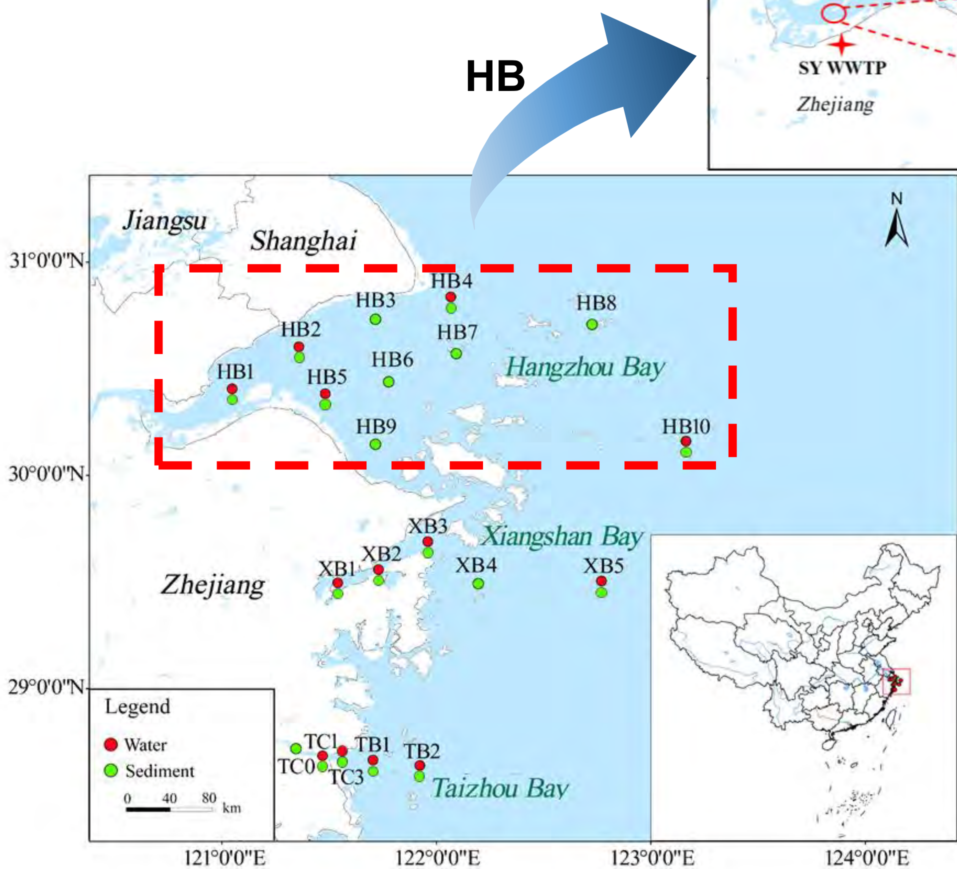
- Coastal area of East China：
  - 13% territory area
  - > 40% population
  - > 60% GDP

- **Water quality of China Seas:**
  - ✓ In the four China Seas, **East China Sea is listed as the worst sea**, especially Yangtze River Estuary and Hangzhou Bay;
  - ✓ Primary pollutants are inorganic nitrogen and orthophosphate;
  - ✓ Organics and emerging pollutants attract more and more attentions.

In the 21st century, 41% of the ocean subject to medium to very high impact (Halpern, *et al.*, 2008).

Study Area and Sampling Sites



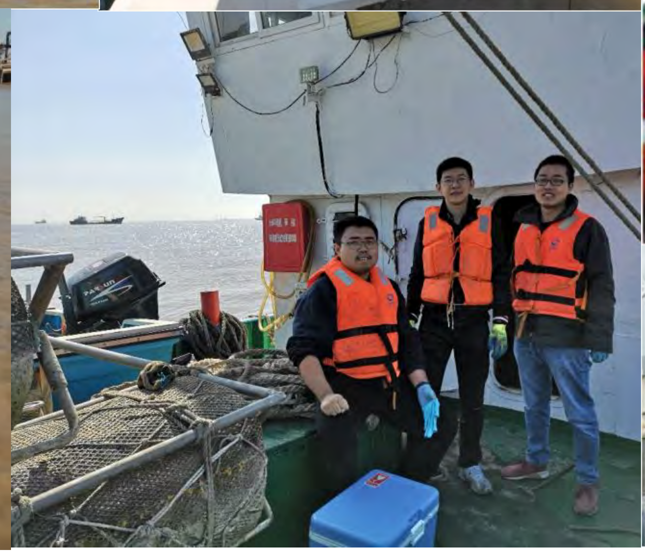


East China Sea:

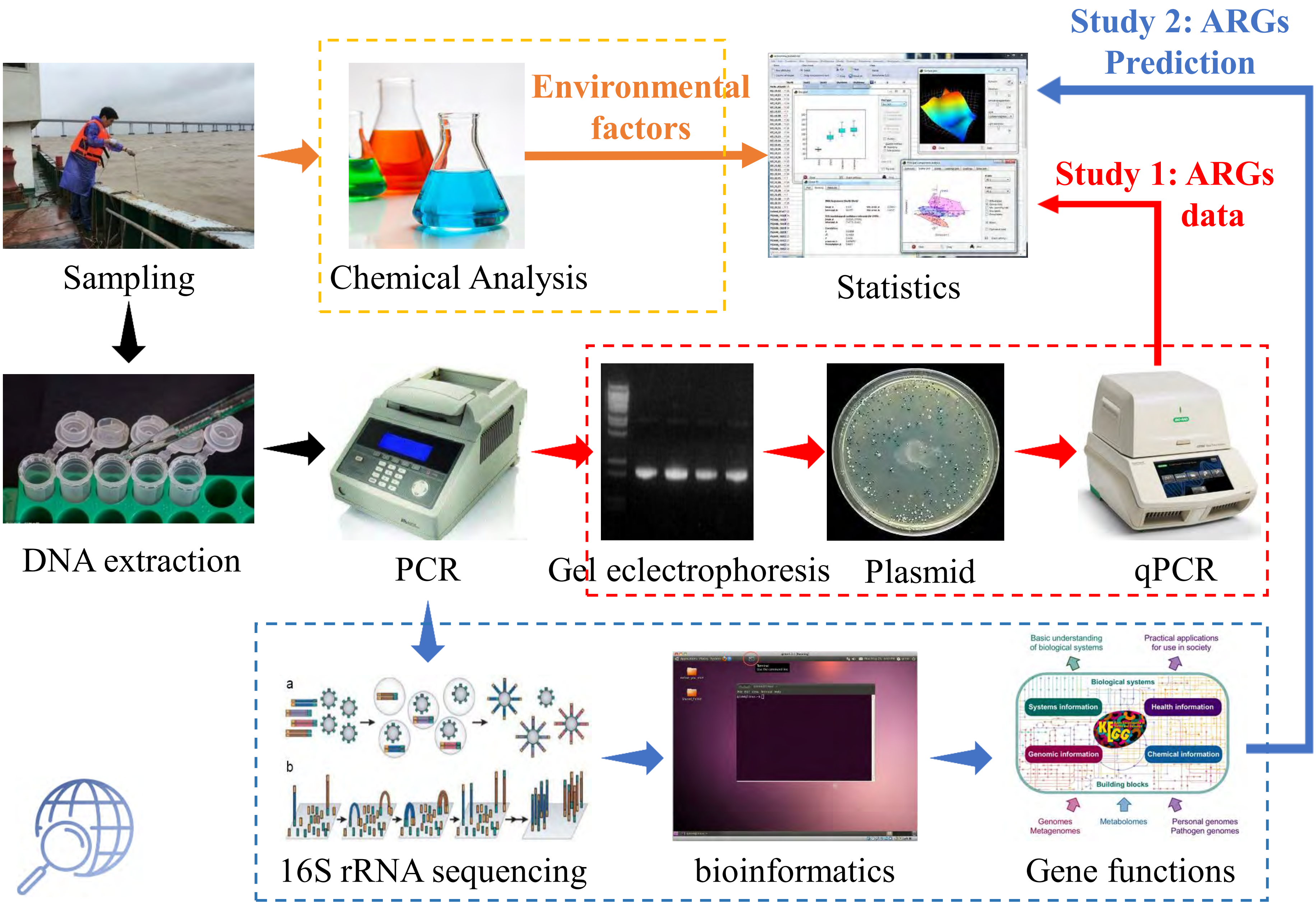
- ✓ HB: Hangzhou Bay (杭州湾)
- ✓ XB: Xiangshan Bay (象山湾)
- ✓ TB: Taizhou Bay (台州湾)

Study Area and Sampling Sites

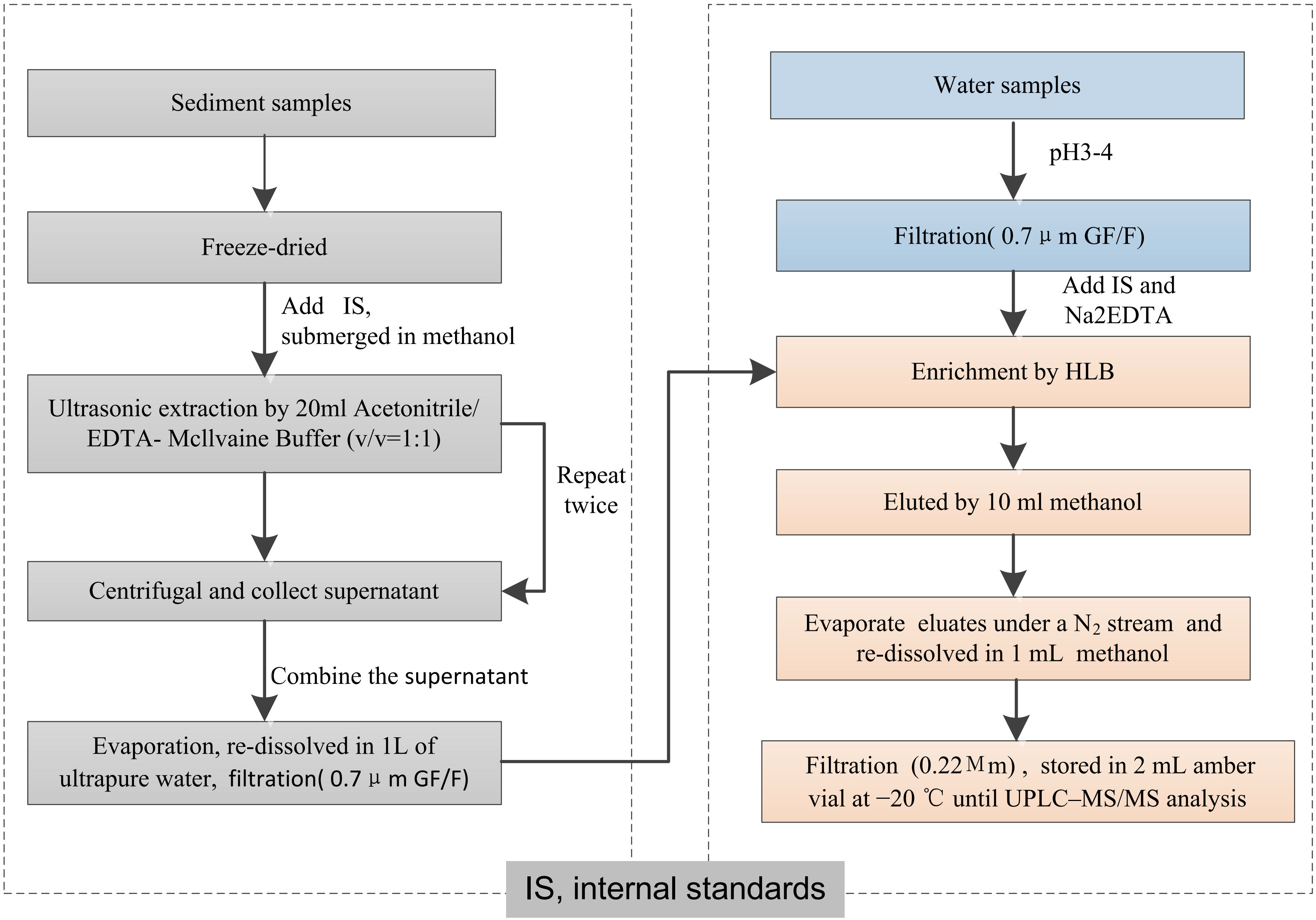



Materials and Methods



Antibiotics Analysis: pre-treatment methods



Antibiotics Analysis

77 Target ARGs:

1) 23 sulfonamides (SAs)
2) 9 macrolides (MLs)
3) 22 quinolones (QNs)
4) 9 tetracyclines (TCs)
5) 12 β-lactams (β-Ls)
6) 2 lincomycins (LMs)

Liquid phase system： ACQUITY UPLC H-Class

Mass spectrometry system： Xevo TQ-S Micro

Mobile phase：A: 0.1% formic acid; B: methanol

Column： Waters BEHC$_{18}$, 2.1×100mm, 1.7μm

ESI+
Capillary：1.0 KV
Desolvation Temp：500℃
Desolvation gas ：1000L/hr

Method 1：SAs，QNs，MLs，LMs

Time	Flow (mL/min)	%A	%B
0	0.3	90	10
1	0.3	80	20
10	0.3	10	90
14	0.3	10	90
14.5	0.3	90	10
18	0.3	90	10

Method 2：β-Ls，TCs

Time	flow(mL/min)	%A	%B
0	0.3	85	15
6	0.3	60	40
7	0.3	45	55
15	0.3	20	80
20	0.3	10	90
20.5	0.3	85	15
25	0.3	85	15

List of Target Antibiotics

23 Sulfonamides (SAs) IS:SMX-D4	Sulfadiazine	SDZ
	Sulfathiazole	STZ
	Sulfapyridine	SPD
	Sulfamerazine	SMZ1
	Sulfamethazine	SMZ
	Sulfamonomethoxine	SMM
	Sulfamethizole	SMIZ
	Sulfameter	SMT
	Sulfachloropyridazine	SCP
	Sulfamethoxypyridazine	SMP
	Sulfadoxine	SDX
	Sulfadimethoxine	SDO
	Sulfamethoxazole	SMX
	Sulfisoxazole	SIZ
	Sulfabenzamide	SBZ
	Sulfaquinoxaline	SQX
	Sulfacetamide	SCM
	Trimethoprim	TMP
	Sulfaphenazole	SPZ
	Sulfamoxole	SMO
	Sulfisomidine; Sulfaisodimidine	SIM
	Sulfaguanidine	SGN
	Sulfapyrazole	SPRZ
9 Macrolides (MLs) IS:RTM-d7	Erythromycin-H2O	ETM
	Roxithromycin	RTM
	Josamycin	JSM
	Tylosin	TLS
	Leucomycins	LCM
	Tilmicosin	TMCS
	Clarithromycin	CLM
	Azithromycin	ATM
	Spiramycin	SRM
2 Lincomycins （LMs） IS:RTM-d7	Lincomycin hydrochloride	LIN
	Clindamycin hydrochloride	CLIN
9 Tetracyclines (TCs) IS:TTC-d6	Oxytetracycline hydrochloride	OTC
	Chlortetracycline hydrochloride	CTC
	Doxycycline hyclate	DXC
	4-Epianhydrochlortetracycline	EHTC

22 Quinolones (QNs) IS:CFX-d8	Enrofloxacin	ERFX
	Norfloxacin	NFX
	Ciprofloxacin	CFX
	Ofloxacin	OFX
	Sarafloxacin	SRFX
	Enoxacin	ENOX
	Lomefloxacin	LMFX
	Nalidixic acid	NAL
	Oxolinic acid	OAO
	Flumequin	FLU
	Danofloxacin	DFX
	Difloxacin	DIF
	Orbifloxacin	OBFX
	Sparfloxacin	SPFX
	Fleroxacin	FLX
	Cinoxacin	CNX
	Gemifioxacin mesylate	GMFX
	Marbofloxacin	MBFX
	Moxifloxacin hydrochloride	MXFX
	Nadifloxacin	NDFX
	Tosufloxacin tosylate	TSFX
	Pipemidic acid	PIP
12 β-Lactam （β-Ls） IS:CPX-d5	Ampicillin	AMP
	Cephalexin	CEPX
	Penicillin G potassium	PEN-Gp
	Oxacillin sodium monohydrate	OXA
	Ceftiofur	CEF
	Amoxicillin trihydrate	Amox2
	Oxacillin	OCL
	Piperacillin	PRCL
	Cefixime	CEFX
	Cephradine	CEHD
	Cefaclor	CEFL
	Cefapirin	CEPR
9 Tetracyclines (TCs) IS:TCT-d6	Tetracycline hydrochloride	TTC
	Minocycline hydrochloride	MCC
	Demeclocycline hydrochloride	DCC
	Methacycline hydrochloride	MTC
	4-Epichlortetracycline hydrochloride	ETC

ARGs Quantification by qPCR

■ Polymerase chain reaction (PCR) of target genes

- ✓ **27** ARG subtypes encoding 8 drug class resistance
- ✓ **16S rRNA**, indicator of microbial biomass
- ✓ ***intI1***, Class 1 integron integrase gene, mediating ARGs horizontal transfer

CFX96 Touch™ Real-Time PCR Detection System (BioRad, USA)

■ Quantitative PCR (qPCR)

11 ARG subtypes were widely detected by PCR in study area and they were selected as our target genes for qPCR

<table>
<tr><th>ARG subtype</th><th>Antibiotic Class</th><th>Resistance Mechanism</th></tr>
<tr><td>tetA</td><td rowspan="4">Tetracycline antibiotic</td><td rowspan="2">Antibiotic efflux</td></tr>
<tr><td>tetC</td></tr>
<tr><td>tetO</td><td rowspan="2">Antibiotic target protection</td></tr>
<tr><td>tetW</td></tr>
<tr><td>sulI</td><td rowspan="2">Sulfonamide antibiotic</td><td rowspan="4">Antibiotic target replacement</td></tr>
<tr><td>sulII</td></tr>
<tr><td>dfrA1</td><td rowspan="2">Diaminopyrimidine antibiotic</td></tr>
<tr><td>dfrA13</td></tr>
<tr><td>bla$_{PSE-1}$</td><td>Beta-lactam antibiotic</td><td>Antibiotic inactivation</td></tr>
<tr><td>floR</td><td>Phenicol antibiotic</td><td>Antibiotic efflux</td></tr>
<tr><td>ermB</td><td>Macrolide antibiotic</td><td>Antibiotic target alteration</td></tr>
</table>

3

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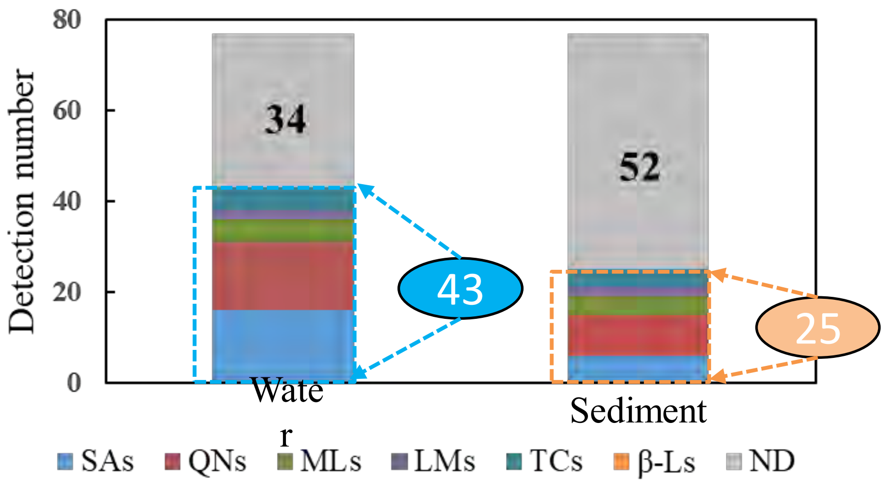
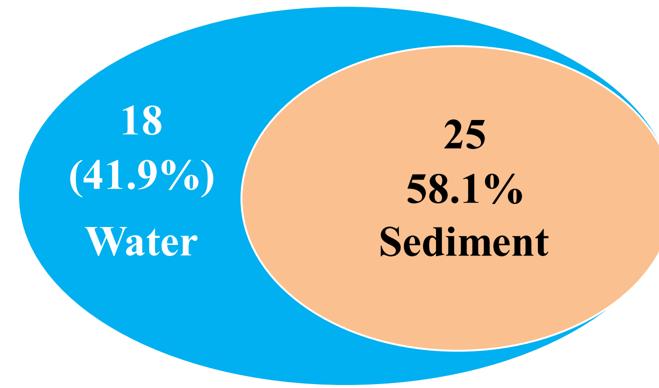
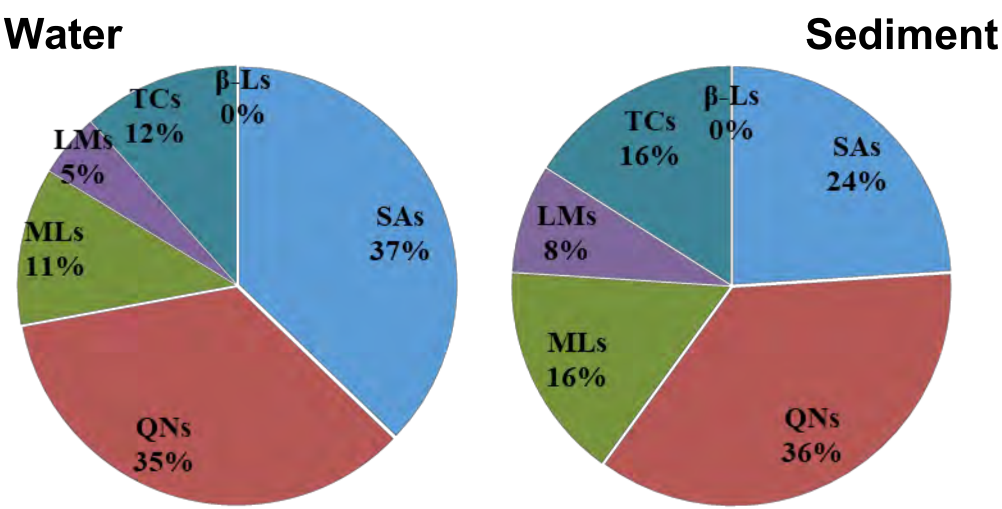
- Study Plan
- **Antibiotics and Risks**
- ARGs and the Risks
- Conclusions

Antibiotic Detection in 3 Bays, 2018



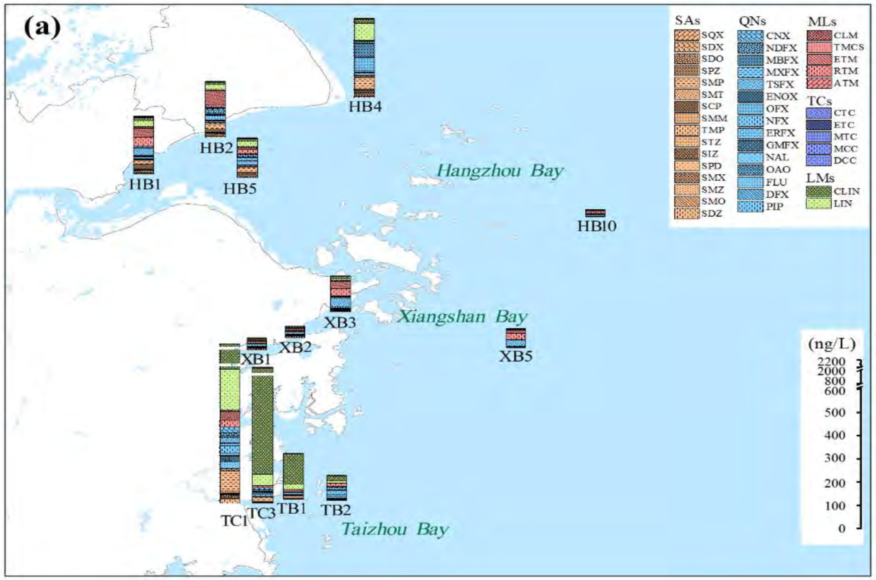


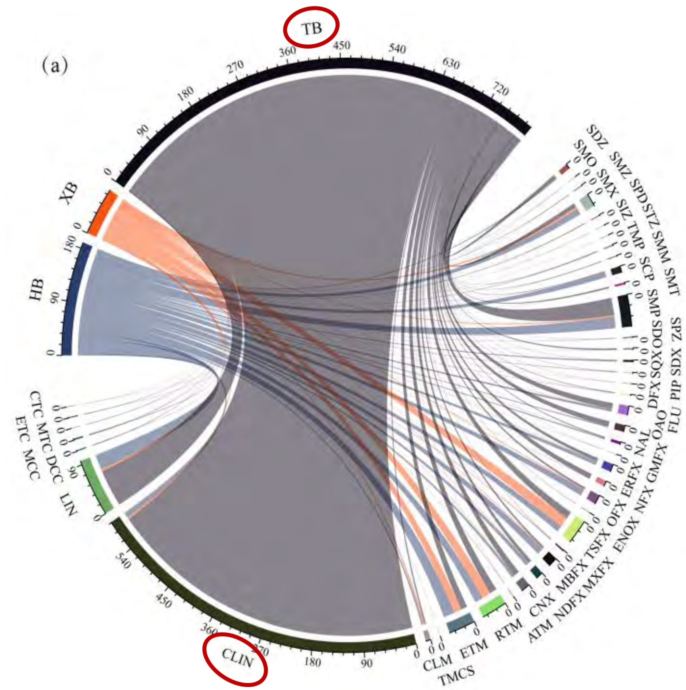


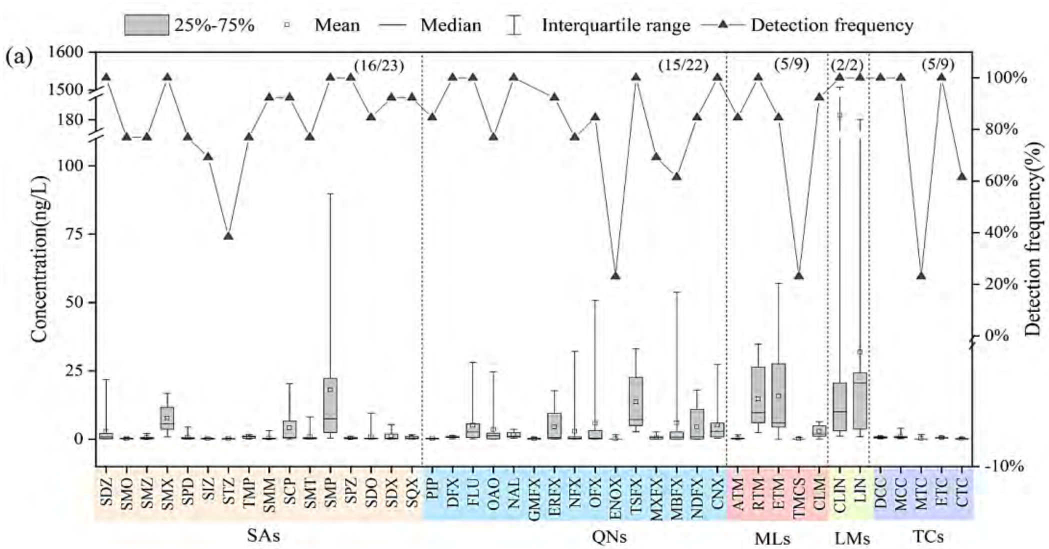
QNs (9)	FLU	LMs (2)	CLIN
	OAO		LIN
	ERFX	TCs (4)	DCC
	NFX		MCC
	OFX		MTC
	TSFX		ETC
	MBFX	SAs (6)	SDZ
	NDFX		SMO
	CNX		SPD
MLs (4)	ATM		SCP
	RTM		SMT
	ETM		SMP
	CLM		

The dominant classes of antibiotics are SAs and QNs in terms of detection number in both water and sediment.

Distribution of Antibiotics in Seawater

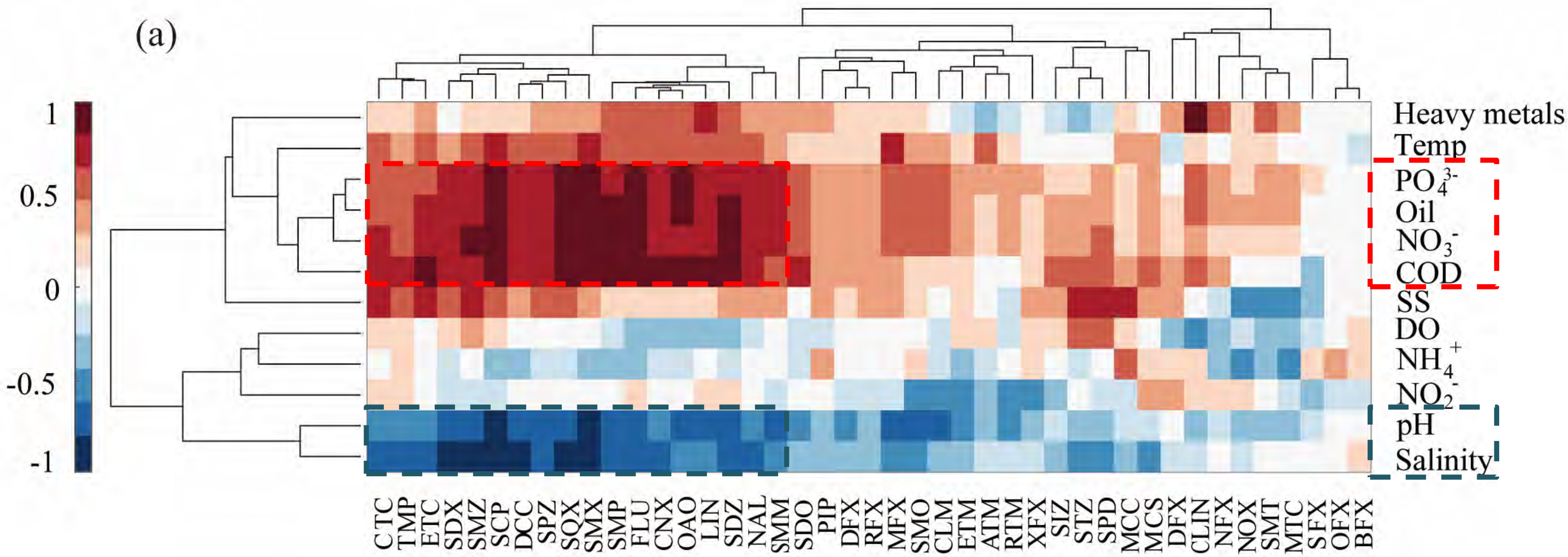






- ✓ **Clindamycin** (Ave.Conc. 183.8 ng/L; 53.2%) and lincomycin (Ave.Conc. 31.8 ng/L; 9.2%) were the top two antibiotics in seawater.
- ✓ **TB** was focused for the most serious antibiotic contamination.

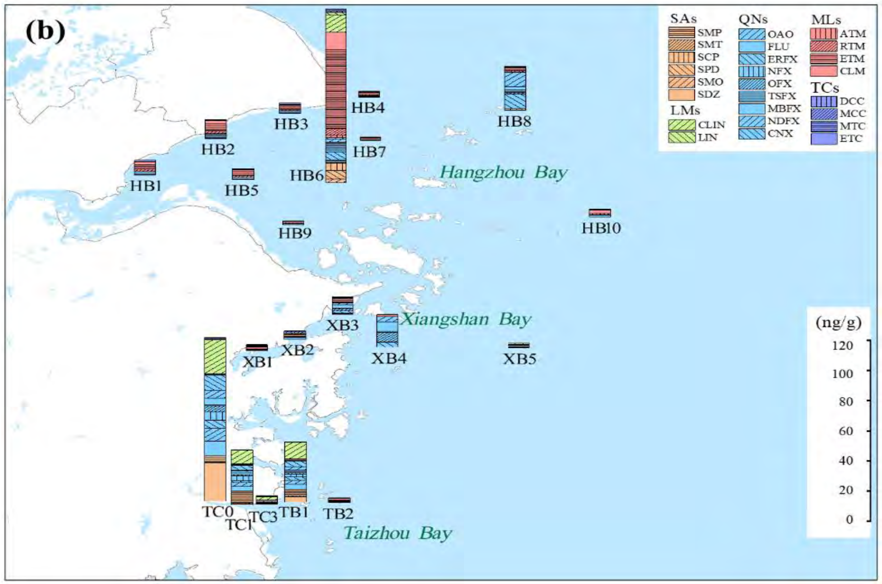
Spearman Correlation
between antibiotics and seawater qualities

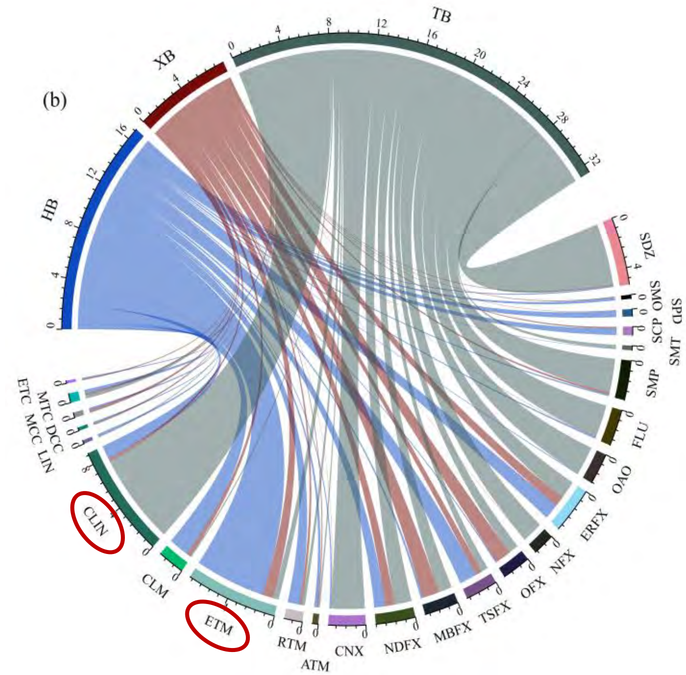


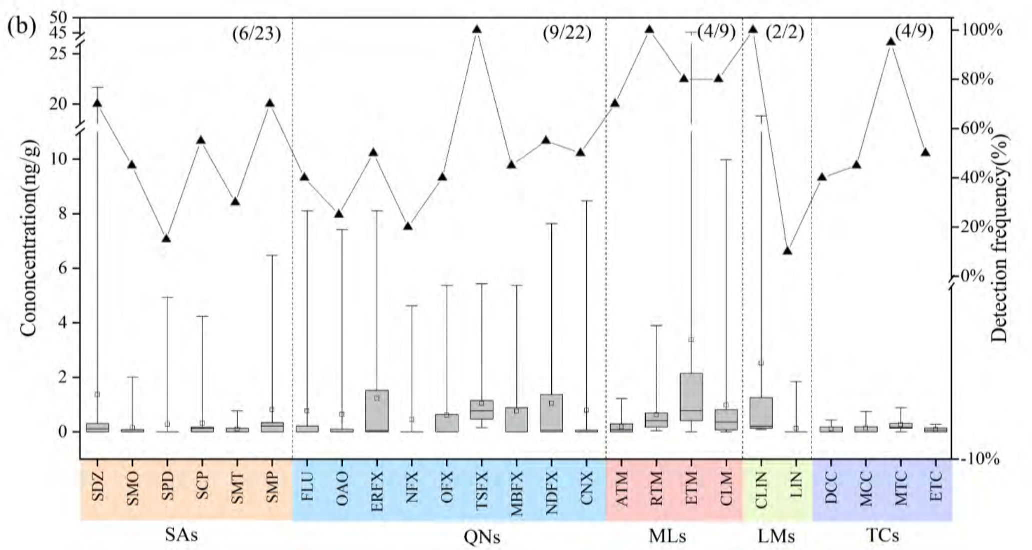
Aquatic antibiotics were

- ✓ **negatively correlated** with natural attribute indicators (**pH and salinity**);
- ✓ **positively correlated** with some anthropogenic pollutants (**oil, PO_4^{3-}, COD and NO_3^-**).

Distribution of Antibiotics in Sediment

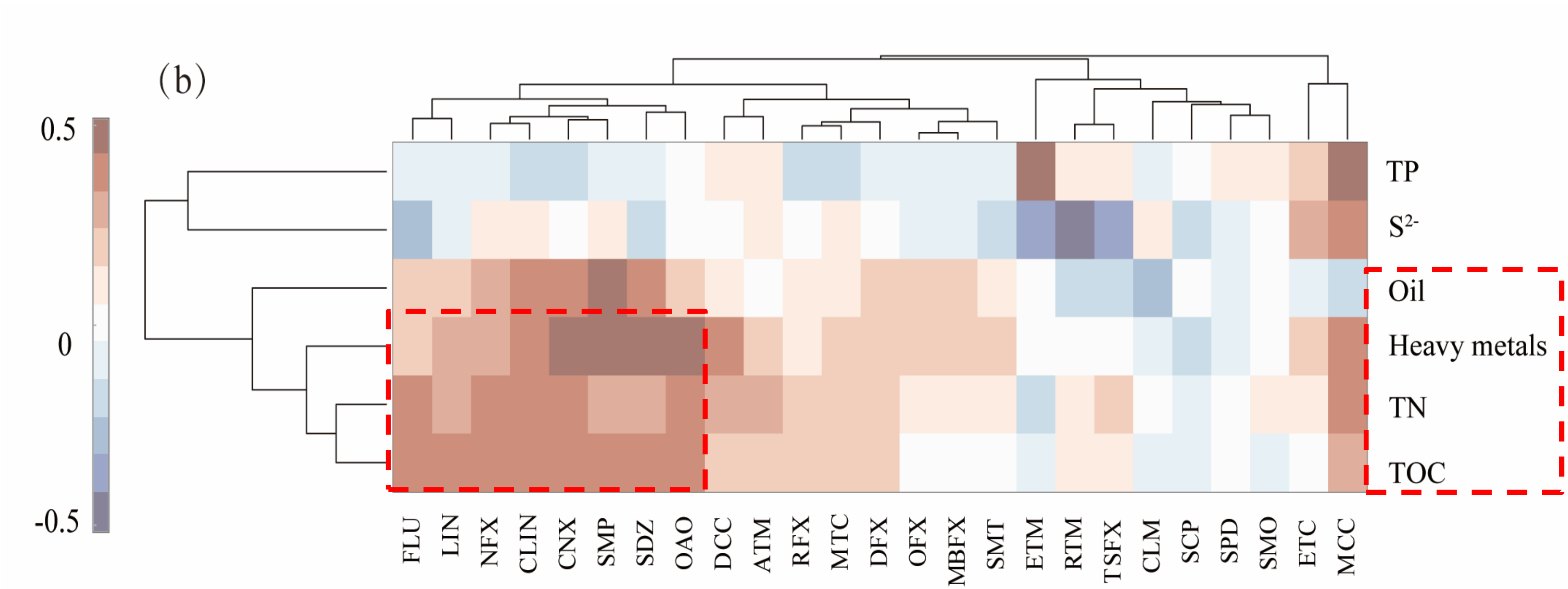






- ✓ **Erythromycin** (Ave.Conc. 3.4 ng/g; 18.1%) and **Clindamycin** (Ave.Conc. 2.5 ng/g; 13.5%) contributed the most to the total antibiotics.
- ✓ **TB6** and **TC0** were the most seriously antibiotic-contaminated sites.

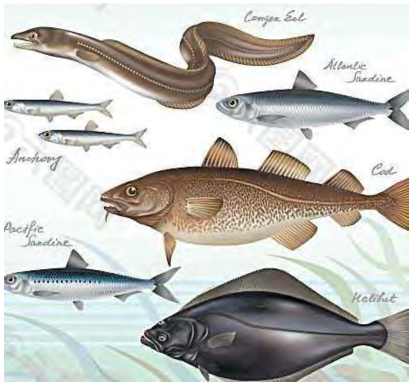
Spearman Correlation
between antibiotics and sediment properties

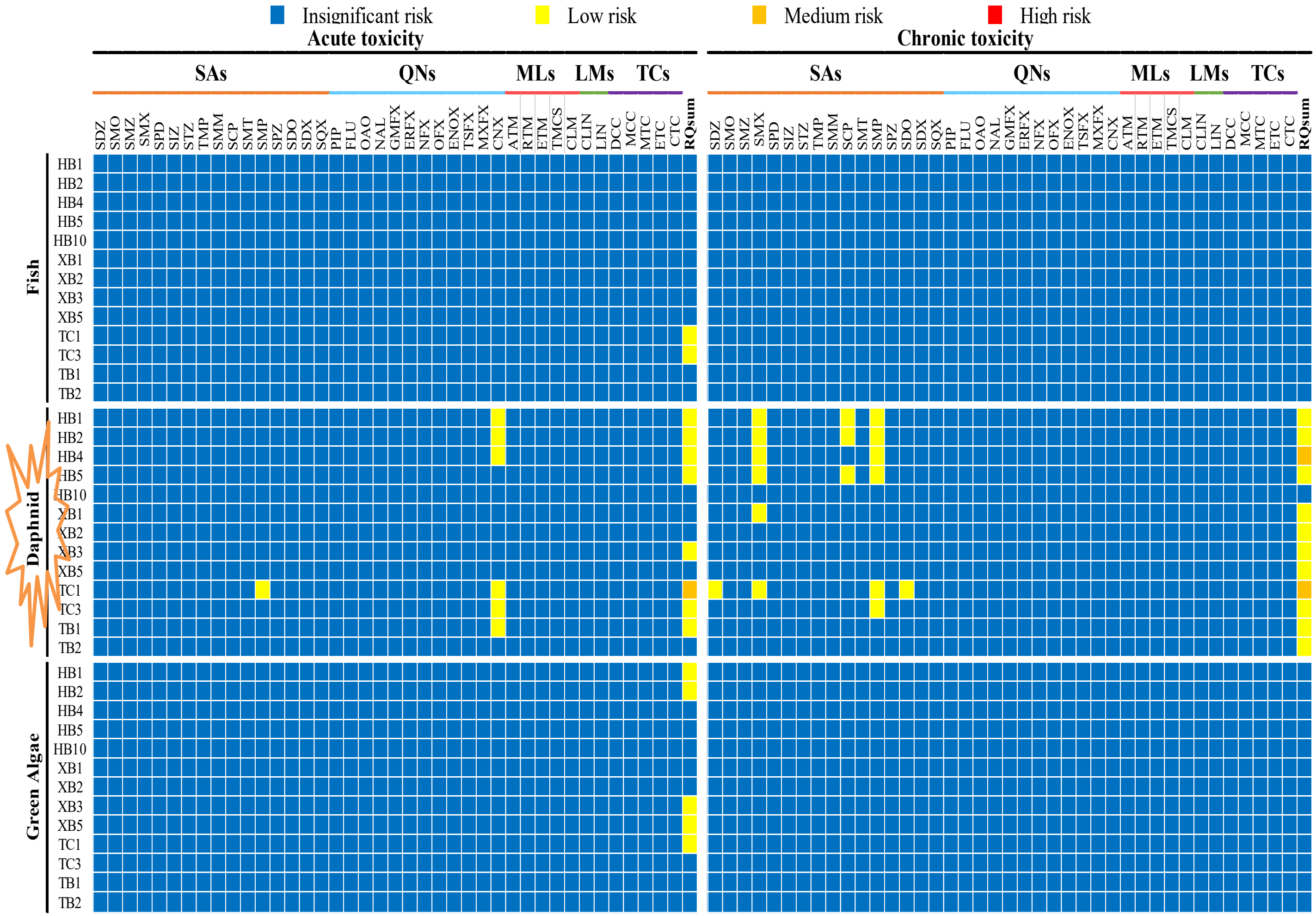


✓ **Positive correlation** between sediment antibiotics and pollutants (**TOC, TN, heavy metals and oil**).

Ecological Risks of Antibiotics

Heatmap of RQ values of antibiotics for aquatic organisms：





✓ SMP, CNX: low acute risks

✓ SDZ, SMX, SCP, SMP, SDO: low chronic risks

Priority Antibiotics in East China Sea

Abbreviation		Names	Use
Sulfonamides:	**SMX ****	Sulfamethoxazole	Human
	SPD	Sulfapyridine	Veterinary
	SCP **	Sulfachloropyridazine	Veterinary
	SMP **	Sulfamethoxypyridazine	Human/Veterinary
	TMP	Trimethoprim	Veterinary
Quinolones:	**CNX**	Cinoxacin	Human
Macrolides:	**ATM**	Azithromycin	Human
	ETM	Erythromycin-H_2O	Veterinary

- ✓ **Priority Antibiotics** in the 3 bays of the East China Sea: in 2017, all the 8 drugs; in 2018, three drugs with **.
- ✓ It is noteworthy that the **joint toxicity** was enhanced when multiple antibiotics were present simultaneously;
- ✓ Fortunately, both the concentrations and ecological risks of the antibiotics decreased as the offshore distance increased.

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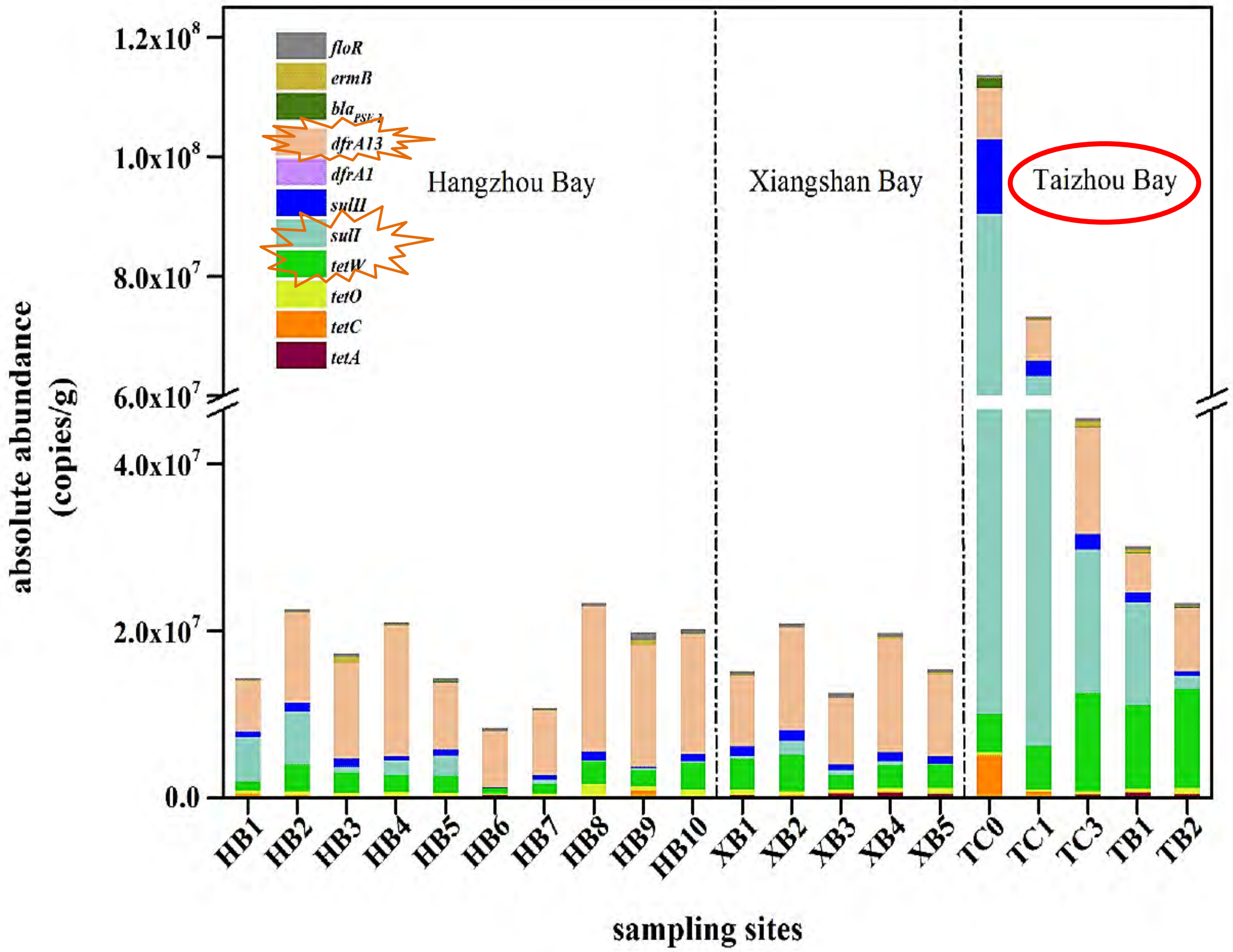
- Background
- Study Plan
- Antibiotics and Risks
- **ARGs and the Risks**
- Conclusions

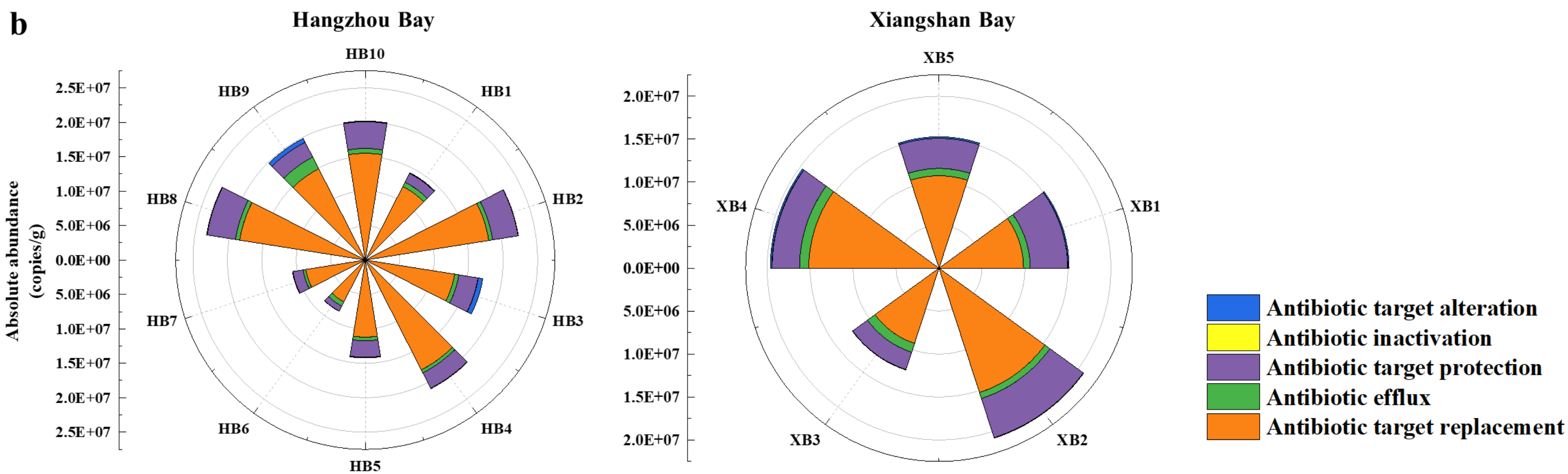
Absolute Abundances of ARGs in Sediment

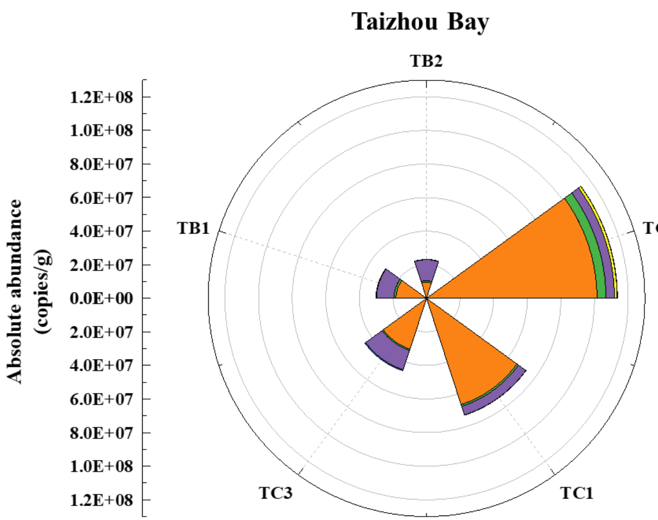


- ✓ TB: the worst bay
- ✓ *dfr*, *sul* and *tet* genes were the most dominant ARG subtypes.

The absolute abundances (TAA) of the detected 11 ARGs in the three bays

Resistance Mechanisms in Sediment



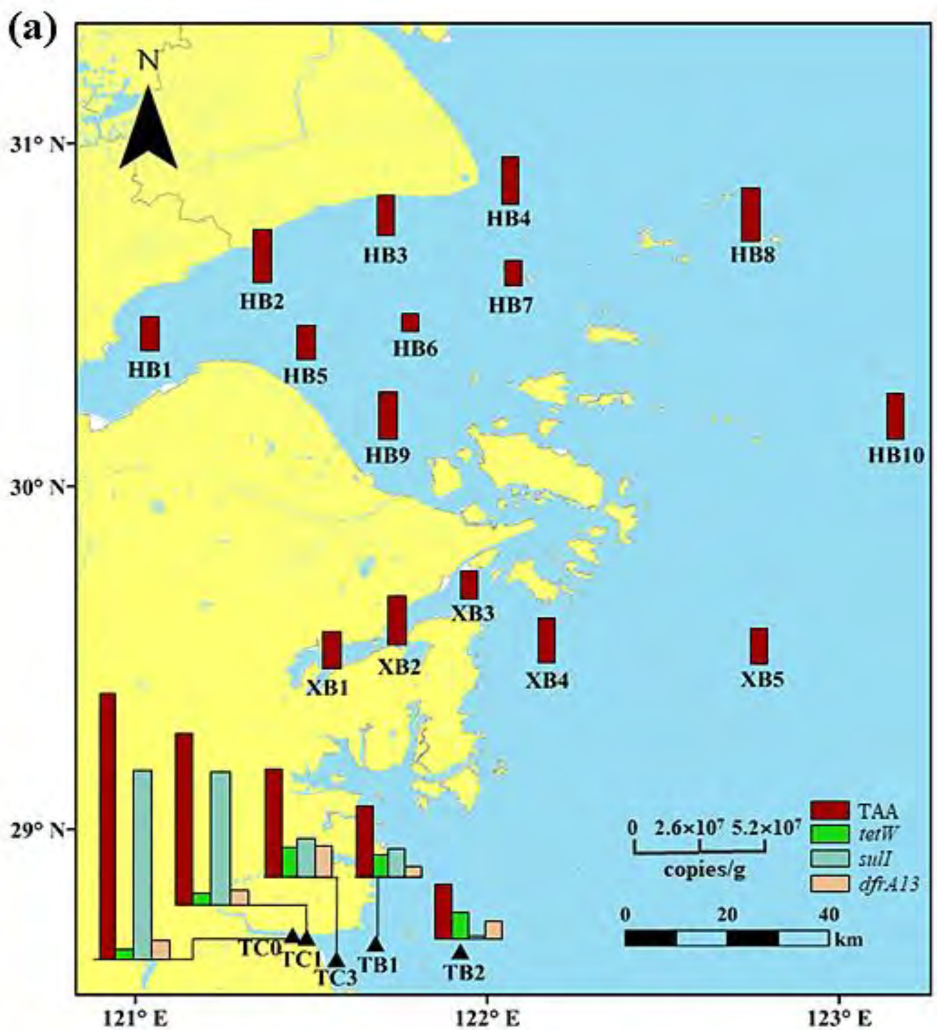


The most important resistance mechanisms:

✓ Target replacement

✓ Target protection

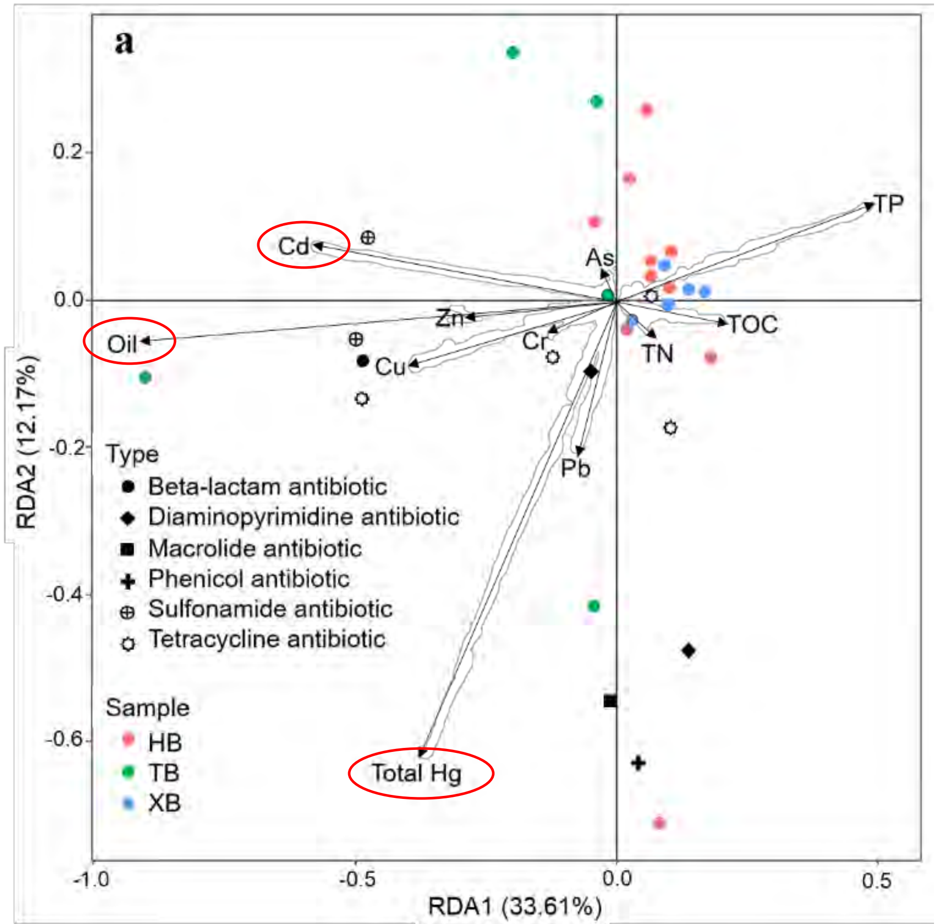
Spatial Distribution of ARGs in Sediment



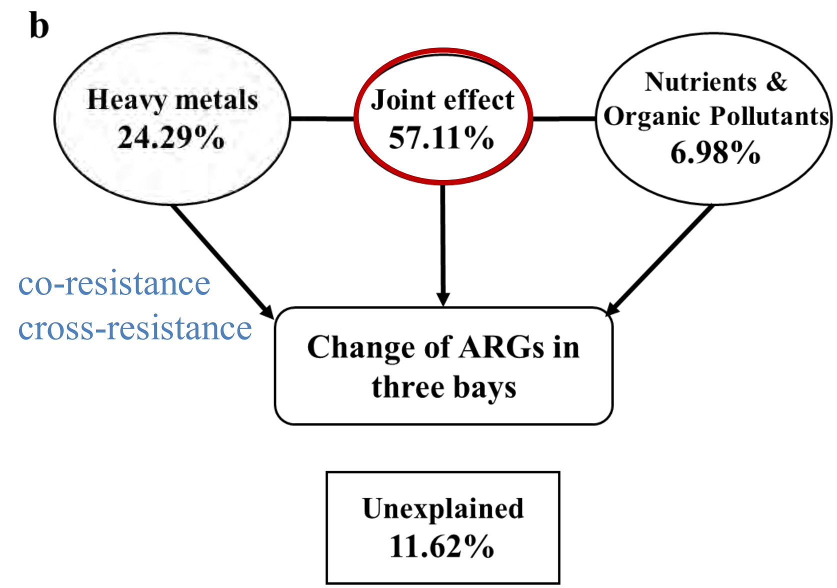
Spatial distribution of dominant ARGs in TB

- ✓ TB： the worst bay
- ✓ *sulI*, *dfrA13*, and *tetW* were the most dominant ARGs.

Relationships between Environmental Factors and ARGs



Redundancy analysis between environmental factors and absolute abundance of ARGs



Variation portioning analysis differentiating the contributions of heavy metals and nutrient substances on the ARGs variation.

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- Background
- Study Plan
- Antibiotics and Risks
- ARGs and the Risks
- **Conclusions**

Antibiotics and their Ecological Risks

1. Antibiotics were proved to be ubiquitous in the coastal marine environment and posed potential threats to aquatic organisms.
2. More than 80% of the detectable concentrations of individual antibiotic were lower than 5.0 ng/L in water and 1.0 ng/g in sediment; nevertheless, the joint toxicity was enhanced when multiple antibiotics were present simultaneously.
3. For the purpose of antibiotics monitoring, a new method was proposed for identifying the priority antibiotics, it might be applied to monitor antibiotics as well as other emerging pollutants in water environment.

ARGs and their Health Risks

1. ARGs (especially Sulfonamide and Tetracycline ARGs) were proved to be ubiquitous in the coastal marine environment and posed potential risks to human health.
2. The spatial distribution and profiles of both antibiotics and ARGs indicated the influence of human activities.
3. Mobile genetic elements (especially integrase genes) promoted the ARGs spread.
4. An effective way of ARGs risk control in coastal areas could be reducing the discharge amount of conventional pollutants from land to the sea.

Thanks for your attention